Bayer CropScience



December 19, 2013

Document Processing Desk 6(a)(2)
Office of Pesticide Programs (7504P)
U. S. Environmental Protection Agency
Room S-4900, One Potomac Yard
2777 South Crystal Drive
Arlington, VA 22202-4501

RE: 6(a)(2) report for alleged bee incidents – Full incident reports for Incident submissions I025484002 through I025484008 (INRI-0199 through 0205)

Dear Sir/Madam:

Bayer CropScience, in response to Mr. Steven Bradbury's July 22, 2013 letter, is submitting information regarding the full details of alleged bee incident investigations for initial notices provided on August 1, 2013. This information represents the final report of these alleged incidents.

Bayer CrocScience R.TP P. O. Box 12014 R.TP, NC 27709 Tel. S19 548-2000

The information with this letter is being submitted to the EPA pursuant to the Agency's interpretation of requirements imposed on registrants by Section 6(a)(2) of FIFRA. This information does not necessarily constitute additional factual information regarding potential unreasonable adverse effects within the meaning of 6(a)(2). It is being submitted to enable the Agency to make its own assessment of the information.

If you have questions or concerns, please do not hesitate to contact me at any time.

Sincerely,

5. Levet Van Duyn

Gerret Van Duyn Compliance Manager State Regulatory and Documentation Services 919-549-2914

CC: Meredith Laws, US EPA Insecticide Branch Chief
AE Coordinator, CA Department of Pesticide Regulation
Jeanine Broughel, NY Department of Environmental Conservation

Final Report

Summary of Alleged Incidents Associated with Corn Planting Dust in the US during the Spring of 2013

Report Number

US0391

Guideline Requirements

None

Author

Iain D. Kelly

Completion Date

November 11, 2013

Submitter:

Bayer CropScience LP

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Research Triangle Park, North Carolina 27709

Summary of Alleged Incidents Purportedly Associated with Corn Planting Dust in the US during the Spring of 2013

Overview

This document provides a summary of alleged bee incidents associated with corn planting dust in the U.S. during Spring of 2013 that were investigated by Bayer. These incidents have been reported to EPA as required under the 6(a)2 Rule.

Background

Prior to 2012, there were very few alleged bee incidents reported in the US associated with corn planting dust. In 2012 Bayer CropScience is aware of approximately 18 alleged incidents. Bayer investigated two of these, one in Indiana and one in Minnesota and concluded that neonicotinoids were a probable contributing factor in the Indiana case but not Minnesota. It is our understanding that a similar picture was observed in the incidents that were not investigated by us but were investigated by State Lead Agencies. Approximately half of these incidents are believed to have indicated no neonicotinoid involvement, with detects of neonicotinoids being low in many of the others.

2013 Investigations

Based on experiences in 2012, we wanted to continue monitoring alleged incidents to help better understand factors that may be contributing to exposure in order to assist in improving stewardship and management practices. We established a Bee Health Investigation Team capable of rapidly assessing and responding as appropriate, to any alleged incidents. In early 2013, prior to corn planting season we met with State Lead Agencies in key mid-West states to outline our proposed program and intent.

In total, eight incidents came to our notice that warranted follow-up action as summarized below.

Overview of Alleged 2013 Incidents

The following table summarizes our investigation of incidents in 2013.





State	Location/ Report No.	Beekeeper description of Incident	Summary of BCS Investigation	
MN	Elbow Lake/ MN-SE-130508	Site was a staging area for a 1,300 hives, Beekeeper reported large number of dead and behaviorally-impaired bees on the ground in front of many of the hives. Onset reportedly began on May 7 when a large field immediately adjacent and on the east side of the bee yard was being planted to corn. The incident was reported to EPA and the MN Dept. of Ag. The Bee Informed Partnership "Bee Squad" based at U. of MN also conducted a parallel investigation.	BCS visited the site on the 9th and 13th of May. Only two cornfields within two miles of the bee yard were identified as having been planted to corn on or prior to May 7, both treated with a low rate (0.25 mg/seed) of clothianidin. Of 19 samples of dead, moribund or behaviorally-impaired bees sampled, 13 were found to contain residues >5 ng/g. Paradoxically, eight of these samples also contained detectable levels of thiamethoxam but there did not appear to be any thiamethoxam use near-by. Willow blossom close to the bee yard did contain clothianidin and thiamethoxam residues but not at high enough levels to cause the observed acute effects. There were few other flowering plants in the area. In conclusion neonicotinoid exposure would appear to be a contributing factor but the source of the exposure is unclear.	-008 Update +0 IO23967 and 4892160
MN	Winthrop/ MN-RH-130515	Beekeeper reported that between May 13 th and 14 th he observed a large number (ca 1000) of dead bees at each of approximately 100 hives at his staging area in Winthrop, MN. He reported that the hives had recently been transported from Texas to Minnesota for the summer and that the colonies appeared to very healthy until the two very windy days above, when he began seeing dead and dying bees. The MN Dept. of Ag and the U of MN Bee Lab both investigated.	BCS visited the site on May 15 and took bee samples from the affected yard. Six of the seven samples had clothianidin residues > 5 ng/g and one had a trace of thiamethoxam. Based on this a follow up visit by BCS and Dr. Rick Fell from VA Tech was conducted between the 20 – 22 May. The commercial operation is extensive with bees being brought into 2 staging areas and distributed to about 170 out-yards. The two staging areas and 4 out-yards were investigated during this second visit. No detectable residues of clothianidin or thiamethoxam were found in dead bees collected during this visit and losses did not seem excessive. Clothianidin and thiamethoxam were found in stored pollen but the source of the exposure is unknown. While some bee health management issues were observed, the overall quality of the equipment and the condition of the majority of hives was good. While it does appear that there were bee losses with neonicotinoid residues being a contributing factor this appears to have been limited over a short time period.	LOOZ Update 1025271- 001 1025530 001 1025484- 003
IN	Lafayette/ IN-MY-130513	Beekeeper reported losses at two of three hives in early May following corn planting in surrounding fields. IN Dept. of Ag. Investigated.	BCS learned of incident May 14 but were unable to visit until June 5 due to beekeeper priorities and could not, therefore, collect samples of affected bees. As of June 5th no hives had been lost.	-001
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		Updates I022340-00	97
		3	Updates IO22340-00 IO25484-00 IO25875-00 IO24495	73

State	Location/ Report No.	Beekeeper description of Incident	Summary of BCS Investigation	
IN	North Manchester/ IN-DS-130520	Beekeeper observed piles of 100- 200 dead bees on May 20, at the entrances of each of the 20 hives located at the apiary and suspected corn planting in nearby fields as the cause. Beekeeper had not visited the apiary in the three weeks prior to the observation and the date of corn planting is uncertain.	BCS visited on May 21 and confirmed observation of dead bees but saw no behaviorally impaired ones. Following an in-hive observation beekeeper considered that the colonies were weaker than hives at his other apiaries. BCS analyzed two samples of dead bees. One contained 11.3 ng/g clothianidin. The other contained only a trace amount of clothianidin. Clothianidin exposure was likely a contributing factor to mortality at some of the hives but due to lack of knowledge of timing of incident little more could be concluded.	1023902-001
IL	Rantoul/ IL-RN-130520	Beekeeper reported large number of dead bees at a total of 8 hives located among three apiaries. Bee losses were observed between 16-18 May with reports of corn planting approximately a day prior to elevated mortalities. IL Dept. of Ag. Investigated.	BCS learned of incident on May 21 and visited on May 22. A sample of dead or dying bees was collected from 2 of the 3 apiaries, containing 10.6 and 4.4 ng/g of clothianidin. Clothianidin exposure was likely a contributing factor to mortality. Colonies appeared strong enough to recover.	1025 484 -006
IL	Urbana/ IL-RJ-130522	Beekeeper observed dead and dying bees in front of the 14 hives at her home apiary on May 19 following corn planting that she observed May 18 in adjacent fields.	BCS heard of incident as it had been shared at the May 19 Central Eastern Illinois Beekeeping Association (CEIBA) meeting. Contacted beekeeper and visited on 23 May, collecting two bee samples, one of dead and one of live bees. Bees were observed exhibiting odd behavior, including clinging to vegetation in front of hive entrances. Clothianidin (7.9 ng/g) was found in the dead bee sample but not the live one. Clothianidin exposure was likely a contributing factor to mortality but the beekeeper and BCS representative estimated that the impact on the colony health was minimal and that the colonies would recover.	Lipdates 1025484 -007
fiL	Penfield/ IL-KP-130516	Beekeeper indicated that she had observed large numbers of dead bees on the ground in front of all five of the hives in her apiary. She said that the onset of the observations occurred on May 15, the day following corn planting in fields adjacent to the apiary. Clothianidin and thiamethoxam were found in dead bees. Evidence is consistent with the beekeeper's report that bees were exposed to abraded	BCS notified of incident by beekeeper on May 16 and visited on May 17. Collected one sample of freshly dead and dying bees in front of one of the	-003 4 date to 1025208-001 1025484-004

corn seed dust.

State	Location/ Report No.	Beekeeper description of Incident	Summary of BCS Investigation
	Maple Park/ IL-WK-130530	Beekeeper pollinated 20-25 acres of apples employing 52 hives. On May 8, it was reported that an adjacent field was being planted to corn and a large cloud of dust was blowing from the field into the orchard. Bee mortality reportedly occurred at the hives in the orchard within 24 hours of this event. IL Dept. of Ag. investigated and apparently estimated 33% total bee loss but anticipated a full recovery.	BCS learned of the incident on May 29 well after the event and too late to collect samples. BCS visited the site on June 14 and contacted the beekeeper on June 25. At that time the beekeeper reported the hives had completely recovered.

Summary

Of the above 8 incidents investigated, Bayer CropScience was made aware of six of them in time to collect meaningful samples of dead or dying bees. The analysis of these samples indicated that exposure to neonicotinoid residues was likely a contributing factor to some bee losses. The losses, however, appeared to occur over a very limited time period and did not appear to put colony survival at risk. The investigations did highlight the difficulties in tracking down the source of exposure retrospectively and indicates the importance of designed studies that are ongoing to better define the source of exposure and the effectiveness of methods being introduced to further reduce any potential exposure.

Iain D. Kelly, Ph.D. Technical Issues Manager. November 11, 2013

Q D Kelly

Final Report

Final Report

Investigation of a May 13, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticidetreated Maize Seed near Lafayette, IN

7025875-003

Report Number

-130513

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

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1.0 Background
On May 14, 2013 lain Kelly of Bayer CropScience (BCS) was contacted by Donna Former of Monsanto regarding bee loss at two hives owned by of Lafayette, IN. bee loss occurred in early May. reported seeing dead and dying bees two days following corn planting in the fields surrounding his property. Mark O'Rourke, of BCS, visited the apiary on June 5 to speak with and view the affected apiary. The apiary consists of three hives located at workshop. The property also serves as the location of the landscaping and lawn care business operated by son. also manages four hives at two additional apiaries. Bee samples were collected by the Indiana State Chemist.
2.0 Investigative Actions
2.1 Field Methods

Planting information was not obtained from the farmer. was reluctant to provide contact information for the grower.

Mark O'Rourke visited the site on June 5. No sample was collected for residue analysis because there were not dead bees remaining that would have provided quality information. Mr. O'Rourke viewed and

3.0 Results

3.1 Results of Survey of Surrounding Landscape

photographed the affected hives (Figure 1).

son operates a lawn care and landscaping business headquartered on the same property as workshop and apiary (Figure 2). Mr. O'Rourke discussed the possibility that there could have been some exposure to his bees from this operation. In noted that he could not think of any practice the lawn business was doing that would have affected his bees at the time of the observed bee loss. Son also plants corn and soybeans on his property adjacent to the workshop and hive area and uses a John Deere MaxEmerge finger pick-up system.

3.2 Observations of Bee Mortality and Behavioral Impairment

Mr. O'Rourke did not observe the reported mortality because of the long time period elapsed between his visit and reported losses.

3.3 Pesticide Residue Analysis Results

Results from samples collected by the Indiana State Chemist have not yet been shared with BCS.

3.4 General Health Status of the Hives Involved

As of June 5th had not experienced any total hive losses.

4.0 Discussion

While it is reported that experienced losses coincident with corn planting in adjacent fields, it is unknown if corn planting contributed to the bee mortality. Without results from residue analysis of bees that died during the mortality event, it is not possible to draw conclusions regarding the effects of any seed treatments.

Figure 1. Photos of reported affected hives



Hive on Left reported to be affected



Front view of one Hive reported to be affected



Hive on Left reported to be affected

Figure 2. apiary and surrounding area



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Final Report

Investigation of a May 13, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticide-treated Maize Seed near Winthrop, Minnesota

Report Number

MN--130515 1025530-001 1025530-001 10255484-003

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

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1.0 Background

Katie Lee of the University of Minnesota contacted Dr. David Fischer, of Bayer CropScience (BCS) on May 15, 2013 regarding a reported bee kill incident that occurred on May 13th and 14th at an apiary in Winthrop, MN. reported a bee kill with over 1000 dead bees at each of approximately 100 hives at his reported that the hives had recently been transported from Texas to Minnesota for the summer and that the colonies appeared to very healthy until two very windy days (May 13-14) when he began seeing dead and dying bees. He reported that the worker bees were dead or gone and that only newly hatched bees appeared to be in the hive. In addition, he saw lethargic or twitching bees on the ground of the bee yard. agreed to allow BCS personnel access to his bee yards for the purpose of investigating the incident. Mark Wrucke, of BCS, investigated the site May 15th and 16th. Further visits were conducted by a team lead by Dick Rogers, BCS apiologist, on May 19th - 22st including Dr. Richard Fell, Professor Emeritus at Virginia Tech, who was invited to conduct an independent investigation. reported the incident to the MN Department of Agriculture.. The MN Department of Agriculture and Judy Wu, University of Minnesota Bee Lab, were on site on May 15th. A copy of Dr. Fell's investigation is included as Appendix A.

2.0 BCS Investigatory Actions

2.1 Field Methods

Two separate visits to the incident site were made by BCS personnel. Mr. Mark Wrucke, BCS Regional Technical Services Manager visited the site on May 15 and collected samples of dead and live but behaviorally-impaired bees in front of several hives scattered throughout the bee yard. He also collected a sample of water from the pond located at the bee yard.

On May 20^{th,} Dick Rogers, Sarah Myers, Dennis Scott and Pat Freeseman, all of BCS, and Dr. Richard Fell of Virginia Tech visited the site to conduct a more detailed follow-up investigation. Mr. Rogers had suggested to that as part of the investigation he allow Dr. Fell, a bee health expert, to inspect general health status of his hives including colony strength, amount of stored food, queen status, presence and levels of parasites and pathogens (virus loads, European foulbrood, etc.). Dr. Fell and Ms. Myers assisted Mr. Rogers with the collection of live bees, honey, fresh and entombed pollen and wax from hives that were assessed for levels of pesticide residues. In addition, Ms. Freeseman collected soil and of bees near the syrup truck stationed at the

Dr. Fell examined 28 hives at five apiary sites including the staging area at extraction and storage facility in Winthrop, MN and four out-yards (Figure 1). The out-yards were investigated because the hives involved in the May 13 and 14 bee kill had been moved to the out-yards at the time of the investigation.

A second staging and transfer area was identified after the initial investigation. The site, indicated as "Transfer site investigation" is a wooded, rural holding area near Gaylord, MN . hives are unloaded from semis at this site following transport from Texas before being distributed to approximately 170 out-yards, including the taging area.

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Mr. Wrucke and George Simkins, also of BCS, contacted the growers who had planted the corn fields within approximately 2 km of the affected bee yard and the Gaylord transfer area and obtained information about the planting dates, corn seed varieties and associated seed treatments that had been planted.

Upon collection, samples were placed in field coolers containing dry ice, and were shipped frozen via overnight express courier service to the BCS Residue Analysis Lab in Research Triangle Park, North Carolina.

Pertinent daily weather data (temperature, humidity, rainfall, daily wind conditions, etc.) recorded at a weather station at a small airport approximately 20 miles northeast of the bee yard were obtained from wunderground.com.

2.2 Laboratory Analysis

Samples were received frozen by the BCS Analytical group in Research Triangle Park, North Carolina and kept frozen until analysis. To prevent cross contamination, all samples were weighed into individual centrifuge tubes containing zircon beads. The samples were extracted with a modified QUECHERS method using a MiniLys bead mixer. Sample extracts were analyzed by high resolution LC/MS-MS, with quantitation against isotopic internal standards added to each extract. The analytical method quantified the levels of clothianidin, TZNG (a degradate of clothianidin) and thiamethoxam. Clothianidin and thiamethoxam are the main insecticidal active ingredients applied as corn seed treatments. In addition, samples were screened for presence of several other insecticide and miticide active ingredients including imidacloprid, carbaryl, chlorpyrifos, atrazine, fluvalinate, coumaphos, amitraz, and several degradates of these.

3.0 Results

3.1 Weather Conditions Prior to and During the Incident

Temperatures during the week of May 10th-16th were unusually high, with temperatures reaching 97°F on May 14, the second day of the observed elevated mortality (Table 1, Figure 2). Observation of high winds on May 13th and 14th was corroborated by weather station records, which show that May 11-14 were marked by high winds, with gusts up over 20 mph all four days. Winds blew from the southeast during the daytime hours on May 13th, shifting to the south in the afternoon. Wind speed was 10-15 mph during the daytime with gusts reaching 28 mph in the afternoon. On May 14th winds shifted throughout the day starting in the west in the early hours, shifting to the south and east through the morning, and blowing from the west in the afternoon. Winds started out calm in the morning and reached sustained speeds of 20-30 mph by mid-afternoon (Figure 2). Very little measurable rainfall or other precipitation occurred in the three days preceding and following the incident (Table 1).

3.2. Results of Survey of Surrounding Landscape

Seventeen cornfields within 2 km of the were identified as having been planted to corn on or prior to May 14 (Figure 3a, Appendix B). There were no

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flowers attractive to bees in any of the nearby fields. The site is located in town with the nearest planted fields about 1 km away. The yard is located next to extraction and storage facility with a small pond, an old tanker truck bed for the storage of corn/sugar syrup for feedings bees (Figure 4a-f.) Few blooming plants were present at the site (only about 10-20 blooming dandelions) (Figure 4g). There were a number of new tree plantings with sawdust piled around the bases (Figure 4h). This area is used as a staging area for moving hives out to summer field locations and is in a constant state of flux. Pallets containing four hives were stacked in groups of three (12 hives per stack) and six stacks (72 hives) were present May 20th and it is likely that the hives present on May 20 were different than the hives present May 13th or 15th.

A second transfer area was identified south of Gaylord, MN. It is likely that all of hives were transported from Texas on semi-trailers and unloaded at the Gaylord transfer area for up to 10 days before moving to the ward. The site is wooded and rural. Thirteen cornfields within 2 km of the bee yard were identified as having been planted to corn on or prior to May 14 (Figure 3b, Appendix C).

3.3 Observations of Bee Mortality and Behavioral Impairment

Dead and behaviorally-impaired bees were observed by both Mr. Wrucke on May 15 and Mr. Rogers' group May 20-22. Wrucke estimated that 90% of the 100 hives in the bee yard on May 15th showed evidence of elevated mortality, with up to 1000 dead bees in front of a given hive (Figure 5). All of the hives were alive. Mr. Wrucke observed numerous bees near the hives and on the ground of the bee yard that were lethargic or twitching. On May 20-22 Rogers and Fell observed a small number of behaviorally-impaired twitching bees at the Lafayette bee yard but there were no large masses of dead or dying bees in front of any of the hives. No elevated mortality or impaired bees were observed at the Transformer, Roflen and Tithel out-yards (Appendix A).

3.4 Pesticide Residue Analysis Results

Results of analysis of samples for pesticide residues are given in Tables 2 and 3.

The threshold lethal dose for clothianidin and thiamethoxam in honey bees is about 1 ng/bee and the LD $_{50}$ dose for oral exposure is about 4 ng/bee. Since individual bees weigh approximately 100 mg, the theoretical concentration expected if a bee ingests a potentially lethal dose is >10 ng/g while a LD $_{50}$ dose should produce a residue of about 40 ng/g. These calculations do not take into account any metabolism or degradation occurring between the initial dosing and the measurement of residues. Past investigations of bee mortality incidents believed to be caused by exposure to clothianidin-laden dust have generally found residue levels to be greater than 5 ng/g in dead bees sampled from affected hives (Pistorius et al. 2009).

Investigations of honey bee incidents performed by the UK government compare measured residues of chemicals in dead bee samples to a subsequent residue level (SRL). These SRL's are determined by measuring the residues of bees dosed at the level of the LD50 in the laboratory. SRL's reported by Grieg-Smith et al. (1994) range from 1.7 to 20% of the applied dose. Laurino et al. (2011) dosed honey bees with clothianidin at several levels and then measured the resultant residues. The lowest dose tested, 3.28 ng/bee resulted in 87% mortality at 48 hr and a clothianidin residue of 0.8 ng/bee. This residue is 24% of the administered dose and is similar to the range

reported by Grieg-Smith (1994). Assuming a honey bee body mass of 0.128 g, this results in a concentration of 6.25 ng/g.

Based on the above, the occurrence of clothianidin residues greater than 5 ppb in dead bees can be interpreted as confirmatory evidence that clothianidin contributed to the mortality observed.

Samples of dead and live bees collected May 15 at the paper appear appear all had measureable residues of clothianidin and the TZNG metabolite. Clothianidin residues ranged from 3.1 to 15.7 ng/g. None of the live bee samples collected on May 21 had any measurable residues of either clothianidin or its TZNG metabolite. Amitraz and coumaphos were detected in some samples, consistent with the use of these insecticides to control mites. Atrazine, thiamethoxam, and imidacloprid were measured in a few of the samples, but not at levels expected to contribute to mortality.

None of the water samples had measurable levels of the pesticides assessed for in the analyses. Two of the three soil samples had levels of clothianidin right at the limit of detection (0.5 ng/g). Imidacloprid was detected in one soil sample at 8.7 ng/g. Low levels of atrazine were detected in all soil samples. Two of the soil samples had amitraz residues at 30 and 118 ng/g.

Pollen samples collected from all five of the visited apiaries on May 21 had measured residues of clothianidin (7.2 to 142.2 ng/g) and thiamethoxam (1.4 to 9.4 ng/g). The TZNG metabolite which results from a N-demethylation of clothianidin was not present in the pollen likely due to the lack of active metabolism in this matrix. Imidacloprid was present in one fourth of the pollen samples with low levels ranging from 0.9 to 1.3 ng/g. Amitraz, coumaphos, and atrazine were also detected. Two samples of entombed pollen were collected; however, neither sample had detectable neonicotinoid residues.

3.5 General Health Status of the Hives Involved

All of the hives were alive, although the health of the hives varied considerably. Evidence of potentially serious honey bee diseases or hive management issues were observed in all of the bee yards. These included diseases (European Foulbrood, chalk brood, viruses) and queen problems (spotty brood patterns, lack of a queen). The overall quality of the equipment and condition of the majority of the hives was good. Dr. Fell's attached report contains detailed notes of hive health (Appendix A). The hives had recently been transported from wintering sites in Texas and were in high density at the Winthrop site.

4.0 Discussion

Six of the seven bee samples collected in the initial visit to the apiary had clothianidin residues above 5 ng/g indicating that this was a likely causal factor in the observed mortality. Clothianidin is also a breakdown product of thiamethoxam, which was detected in one sample. The presence of clothianidin could be indicative of initial exposure to either of these insecticides.

Both clothianidin and thiamethoxam were detected in all of the pollen samples collected from the five apiaries. This indicates that all of these colonies had exposure to these insecticides. The report of Dr. Fell (Appendix A) states that "None of the hives showed signs consistent with serious pesticide damage, and there was no evidence of significant bee kills at any of the apiary sites." This suggests that the measured pollen levels are at levels below that at which significant elevated mortality occurs.

Recently, the EPA along with PMRA and California DPR, released a risk assessment process for bees (EPA et al. 2012). In this white paper, based on consumption estimates, they conclude that the nurse bees within the hive consume the most pollen and would be the bees most at risk from pesticide residues in pollen. They estimate that a nurse bee would consume 8.85 mg pollen per day. If one assumes that thiamethoxam is equivalent to clothianidin, the highest clothianidin equivalents measured in a pollen samples is 151.6 ng/g. Using values of 3.79 ng/bee as the oral LD50 and a nurse bee consumption of 8.85 mg pollen/d at 151.6 ng/g clothianidin, the calculated risk quotient is 0.35. This risk quotient is below the level of concern of 0.4 established by EPA for presuming a risk from dietary exposure, and is consistent with the lack of toxicity observed at these apiaries.

5.0 References

Environmental Protection Agency, Pest Management Regulatory Agency, California Department of Pesticide Regulation. 2012. White paper in support of the proposed risk assessment process for bees.

Grieg-Smith P.W., Thompson H.M., Hardy A.R., Bew M.H., Findlay E., Stevenson J.H. 1994. Incidents of poisoning of honeybees (Apis mellifera) by agricultural pesticides in Great Britain 1981-1991. Crop Protection 13(8):567-581.

Pistorius J., Bischoff G., Heimbach U., Stähler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Küln-Archiv 423:118-126.

Laurino D., Porporato M., Patetta A., Manino A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. Bulletin of Insectology 64(1):107-113.

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Table 1. Daily record of temperature and precipitation at Glencoe, Minnesota weather station May 1- May 31, 2013

2013	Te	mp. (°	F)	W	ind (m	ph)	Precip. (in)	Events
May	high	avg	low	high	avg	high	sum	
1	52	44	33	20	9	24	0.22	Rain , Snow
2	48	41	34	20	13	28	0.00	
3	41	36	31	18	10	28	0.00	Rain
4	44	38	33	13	9	21	0.00	Rain
<u>5</u>	65	51	38	10	6	-	0.00	
<u>6</u>	73	58	42	7	4	-	0.00	
7	78	62	46	10	3	17	0.00	
8	72	64	57	13	4	17	0.04	Rain
9	62	54	45	15	8	26	0.02	Rain
10	68	52	36	12	7	17	0.00	
<u>11</u>	54	48	39	30	19	37	0.00	
12	60	44	30	16	6	24	0.00	
13	83	60	37	17	4	28	0.00	Rain
14	97	74	50	32	10	39	0.00	Rain
<u>15</u>	79	65	52	17	10	25	0.00	Rain
<u>16</u>	82	66	51	16	6	22	0.00	Rain
17	61	60	56	17	11	21	0.56	Rain
<u>18</u>	81	68	57	21	10	25	1.25	Rain , Thunderstorm
<u>19</u>	78	70	62	16	8	24	1.13	Rain , Thunderstorm
<u>20</u>	71	65	60	17	7	22	0.11	Rain , Thunderstorm
21	62	55	47	9	6	18	0.04	Rain
22	53	48	44	14	9	26	0.20	Rain
23	63	54	44	14	7	24	0.00	
24	69	54	39	16	7	24	0.16	Rain , Thunderstorm
<u>25</u>	58	55	52	23	12	29	0.02	Rain
26	56	52	50	26	14	33	0.00	
<u>27</u>	62	58	54	18	12	23	0.00	Rain
<u>28</u>	60	56	53	13	7	-	0.00	Rain
29	75	64	52	20	6	31	0.06	Fog , Rain , Thunderstorm
<u>30</u>	74	69	64	16	12	24	0.08	Rain , Thunderstorm
31	74	67	61	21	9	28	0.02	Rain , Thunderstorm

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Table 2. Analytical chemistry results - Bees

	,		Concentration in ng/g (ppb)			
Sample ID	Date Collected	Sample type	Clothianidin	TZNG	Thia- methoxam	
W1	15-May	dead bees	15.7	3.5	<lod< td=""></lod<>	
W2	15-May	live bees	7.2	2.2	0.6	
W3	15-May	dead bees	8.7	1.3	<lod< td=""></lod<>	
W4	15-May	dead bees	3.1	1.8	<lod< td=""></lod<>	
W5	15-May	dead bees	15.0	5.3	<lod< td=""></lod<>	
W6	15-May	live bees	7.8	1.2	<lod< td=""></lod<>	
W7	15-May	dead bees	13.0	2.3	<lod< td=""></lod<>	
Live bees collected d	uring Dr. Rick	Fell's investigation				
HonlBees-H101-B	21-May	H101 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H102-B	21-May	H102 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H103-B	21-May	H103 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H104-B	21-May	H104 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H201-B	21-May	H201 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H202-B	21-May	H202 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H301-B	21-May	H301 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H302-B	21-May	H302 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H401-B	21-May	H401 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H402-B	21-May	H402 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H501-B	21-May	H501 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-H502-B	21-May	H502 Bees	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	
HonlBees-15	21-May	Bees near Syrup Truck	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>	

Table 2. Analytical chemistry results – Bees continued

		Concentration ng/g (ppb)				
Sample ID	Amitraz	Total Imidacloprid*	Atrazine	Coumaphos		
W1	Traces	<lod< td=""><td>2</td><td></td></lod<>	2			
W2	Traces	<lod< td=""><td>2</td><td></td></lod<>	2			
W3	Traces	<lod< td=""><td>2</td><td></td></lod<>	2			
W4	Traces	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
W5	Traces	<lod< td=""><td>2</td><td></td></lod<>	2			
W6	Traces	<lod< td=""><td>2</td><td></td></lod<>	2			
W7	Traces	<lod< td=""><td>2</td><td></td></lod<>	2			
Live bees collected during Dr. Rick			4.00			
HonlBees-H101-B	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H102-B	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H103-B	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H104-B	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H201-B	<lod< td=""><td><lod< td=""><td>1</td><td></td></lod<></td></lod<>	<lod< td=""><td>1</td><td></td></lod<>	1			
HonlBees-H202-B	Traces	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H301-B	Traces	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H302-B	Traces	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H401-B	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H402-B	Traces	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			
HonlBees-H501-B	14	<lod< td=""><td>1</td><td></td></lod<>	1			
HonlBees-H502-B	<lod< td=""><td><lod< td=""><td><lod< td=""><td>φ.</td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td>φ.</td></lod<></td></lod<>	<lod< td=""><td>φ.</td></lod<>	φ.		
HonlBees-15	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>			

Limit of detection = 0.5 ng/g for clothianidin, 0.4 ng/g for TZNG (a metabolite of clothianidin), 0.6 ng/g for thiamethoxam, 0.7 ng/g for total imidacloprid, 1.3 ng/g for atrazine, and 1.5 ng/g for coumaphos . Traces = detectable residue less than 10 ng/g. *Total imidacloprid is a total of parent, olefin and 5-hydroxy unless otherwise noted.

Table 3. Analytical chemistry results - Water, Pollen, Soil

Date Collected 15-May	Sample type Water	Clothianidin	TZNG	Thia-
	Water		I ZITO	methoxam
		<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	H101 Pollen	44.9	<lod< td=""><td>6.0</td></lod<>	6.0
21-May	H102 Pollen	142.2	<lod< td=""><td>9.4</td></lod<>	9.4
21-May	H103 Pollen	65.9	<lod< td=""><td>5.7</td></lod<>	5.7
21-May	H104 Pollen	44.9	<lod< td=""><td>5.4</td></lod<>	5.4
21-May	H201 Pollen	8.1	<lod< td=""><td>3.7</td></lod<>	3.7
21-May	H202 Pollen	16.1	<lod< td=""><td>6.9</td></lod<>	6.9
21-May	H301 Pollen	13.9	<lod< td=""><td>2.6</td></lod<>	2.6
21-May	H302 Pollen	53.7	<lod< td=""><td>5.8</td></lod<>	5.8
21-May	H401 Pollen	9.0	<lod< td=""><td>1.4</td></lod<>	1.4
21-May	H402 Pollen	7.2	<lod< td=""><td>1.9</td></lod<>	1.9
21-May	H501 Pollen	24.4	<lod< td=""><td>6.0</td></lod<>	6.0
21-May	H502 Pollen	14.6	<lod< td=""><td>2.0</td></lod<>	2.0
21-May	H102 Entombed Pollen	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	H502 Entombed Pollen	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	Sample 10	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	Honl Water, Sample 11	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	Soil, Sample #10	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	Soil, Sample #11	0.5	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
21-May	Soil, Sample #16	0.5	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
	21-May 21-May 21-May 21-May 21-May 21-May 21-May 21-May 21-May 21-May 21-May 21-May 21-May	21-May H104 Pollen 21-May H201 Pollen 21-May H202 Pollen 21-May H301 Pollen 21-May H302 Pollen 21-May H401 Pollen 21-May H501 Pollen 21-May H502 Pollen 21-May Pollen 21-May Pollen H502 Entombed Pollen Honl Water, Sample 10 Honl Water, Sample 11 21-May Soil, Sample #10 21-May Soil, Sample #11	21-May H104 Pollen 44.9 21-May H201 Pollen 8.1 21-May H202 Pollen 16.1 21-May H301 Pollen 13.9 21-May H302 Pollen 53.7 21-May H401 Pollen 9.0 21-May H402 Pollen 7.2 21-May H501 Pollen 24.4 21-May H502 Pollen 14.6 H102 Entombed Pollen <lod< td=""> 21-May Pollen <lod< td=""> Honl Water, Sample 10 LOD <lod< td=""> Honl Water, Sample 11 <lod< td=""> 21-May Soil, Sample #10 <lod< td=""> 21-May Soil, Sample #10 <lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<>	21-May H104 Pollen 44.9 <lod< td=""> 21-May H201 Pollen 8.1 <lod< td=""> 21-May H202 Pollen 16.1 <lod< td=""> 21-May H301 Pollen 13.9 <lod< td=""> 21-May H302 Pollen 53.7 <lod< td=""> 21-May H401 Pollen 9.0 <lod< td=""> 21-May H402 Pollen 7.2 <lod< td=""> 21-May H501 Pollen 24.4 <lod< td=""> 21-May H502 Pollen 14.6 <lod< td=""> 21-May Pollen <lod< td=""> <lod< td=""> 41-May Pollen <lod< td=""> <lod< td=""> 21-May Pollen <lod< td=""> <lod< td=""> 21-May Pollen <lod< td=""> <lod< td=""> 21-May Soil, Sample #10 <lod< td=""> <lod< td=""> 21-May Soil, Sample #10 <lod< td=""> <lod< td=""> 21-May Soil, Sample #10 <lod< td=""> <lod< td=""></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<></lod<>

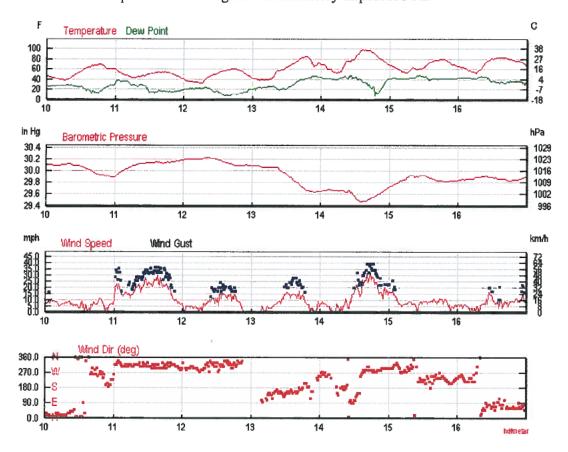
Table 3. Residue analysis results continued – Water, Pollen, Soil

		Concentration	ng/g (ppb)	Γ
Sample ID	Amitraz	Total Imidacloprid*	Atrazine	Coumaphos
W8	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>	
H101-P	Traces	1.3 (parent only)	22	<lod< td=""></lod<>
H102-P	Traces	1.3 (parent only)	26	96
H103-P	Traces	<lod< td=""><td>14</td><td><lod< td=""></lod<></td></lod<>	14	<lod< td=""></lod<>
H104-P	Traces	<lod< td=""><td>10</td><td><lod< td=""></lod<></td></lod<>	10	<lod< td=""></lod<>
H201-P	Traces	0.9 (parent only)	16	4
H202-P	Traces	<lod< td=""><td>16</td><td><lod< td=""></lod<></td></lod<>	16	<lod< td=""></lod<>
H301-P	Traces	<lod< td=""><td>4</td><td><lod< td=""></lod<></td></lod<>	4	<lod< td=""></lod<>
H302-P	24	<lod< td=""><td>11</td><td><lod< td=""></lod<></td></lod<>	11	<lod< td=""></lod<>
H401-P	Traces	<lod< td=""><td>3</td><td>6</td></lod<>	3	6
H402-P	92	<lod< td=""><td>5</td><td><lod< td=""></lod<></td></lod<>	5	<lod< td=""></lod<>
H501-P	Traces	<lod< td=""><td>4</td><td>6</td></lod<>	4	6
H502-P	48	<lod< td=""><td>18</td><td><lod< td=""></lod<></td></lod<>	18	<lod< td=""></lod<>
H102-EP	26	<lod< td=""><td>6</td><td>151</td></lod<>	6	151
H502-EP	78	<lod< td=""><td><lod< td=""><td><lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""></lod<></td></lod<>	<lod< td=""></lod<>
HonlWater-12	<lod< td=""><td><lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td></td></lod<></td></lod<>	<lod< td=""><td></td></lod<>	
HonlWater-13	Traces	<lod <lod< td=""><td><lod< td=""><td></td></lod<></td></lod<></lod 	<lod< td=""><td></td></lod<>	
	110000	-200	-200	
HonlSoil-10	<lod< td=""><td><lod< td=""><td>2</td><td></td></lod<></td></lod<>	<lod< td=""><td>2</td><td></td></lod<>	2	
HonlSoil-11	30	8.7 (parent only)	2	
HonlSoil-16	118	<lod< td=""><td>2</td><td></td></lod<>	2	

Limit of detection = 0.5 ng/g for clothianidin, 0.4 ng/g for TZNG (a metabolite of clothianidin), 0.6 ng/g for thiamethoxam, 0.7 ng/g for total imidacloprid, 1.3 ng/g for atrazine, and 1.5 ng/g for coumaphos . Traces = detectable residue less than 10 ng/g. *Total imidacloprid is a total of parent, olefin and 5-hydroxy unless otherwise noted.



Figure 2. Weather History for Glencoe, MN for May 10, 2013 through May 16, 2013 Data Source: http://www.wunderground.com/history/airport/KGYL/



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Figure 3a. Map of the planting data included in Appendix B.

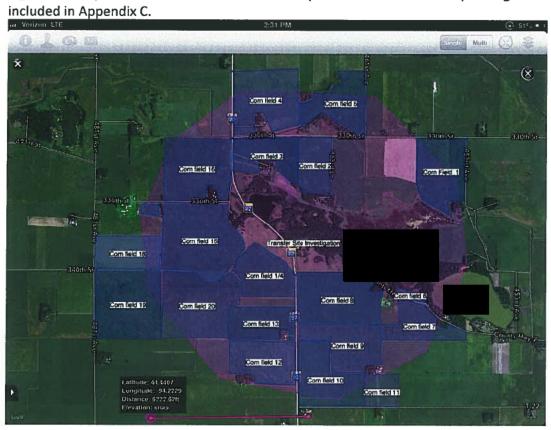
205 PM

2

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Figure 3b. Map of the transfer site in relation to planted cornfields. Field planting data included in Appendix C



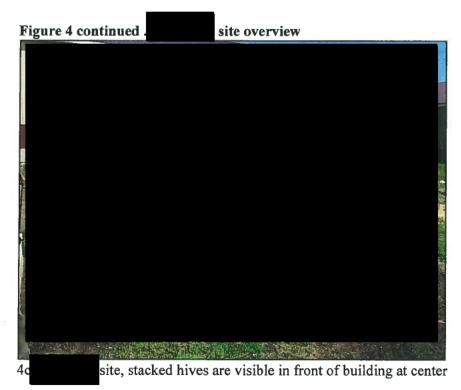


4a. Example of affected Hives



4b. Hives near small pond on site

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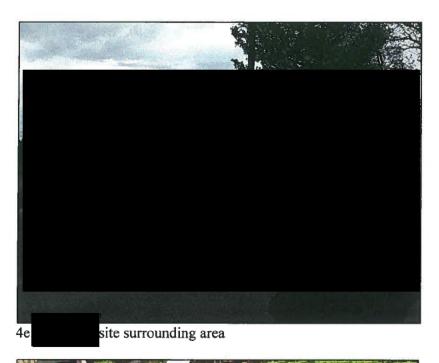




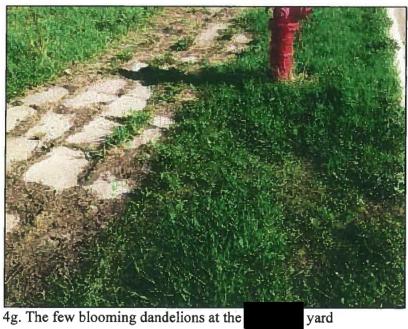
4d. Parked corn syrup tanker

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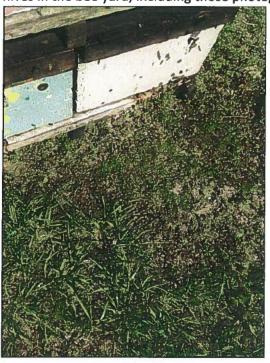


4g. The few blooming dandelions at the



4f. Sawdust around planted trees where dead bees were found

Figure 5. Dead Bees In Front of Hives. While it is normal for some dead bees to be found on the ground in front of bee colonies, the number was elevated at many of the hives in the bee yard, including those photographed below.





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Appendix A. Field investigation summary prepared by Richard Fell, PhD, Professor Emeritus at Virginia Tech

Field Investigation of Reported Bee Kill by Commercial Beekeeper Winthrop Minnesota, May 20-22, 2013

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Submitted by: Richard Fell, Ph.D.

Professor Emeritus, Virginia Tech, Blacksburg VA 24060

Summary: Honey bee colonies were examined at five apiary sites in the vicinity of Winthrop, Minnesota for possible pesticide damage. The sites consisted of a staging area next to s extraction and storage facility and 4 out-yards. A total of 28 hives were examined with samples collected from 12 hives. None of the hives showed signs consistent with serious pesticide damage, and there was no evidence of significant bee kills at any of the apiary sites. Decomposing bees were present at the Winthrop site and could reflect some loss due to pesticide kill. However, the numbers of dead bees were not excessive, especially given the numbers of hives brought in to the yard on a temporary basis before distribution to other apiary sites. A small number of 'twitching' bees (<5) and a very small number of dead bees were found in front of hives but there was no evidence of larger bee kills. Entombed cells of pollen were observed in hives at two yards indicating the likelihood of pollen contaminated with pesticide residues. Possible sources of contamination are unknown and could be local or from previous hive locations (Texas, California). Consumption of contaminated pollen could explain some of the reported bee mortality at the site. Management problems were observed in all of the bee yards and could account for some of the poor colony conditions reported by The management issues included diseases (European foulbrood, chalkbrood, viruses), and queen problems (spotty brood patterns, lack of a queen). On the other hand, the overall quality of the equipment and the condition of the majority of hives was good.

Specific Comments Regarding Apiary Sites

Site: The site is located next to the storage and extracting facilities for the beekeeping operation. Pallets containing 4 hives were stacked in groups of 3 (12 hives per stack) and 6 stacks were present in the yard at the time of our investigation. Higher numbers of hives had been present the previous week, but some had been moved to out-yard locations. In addition to hives on pallets in the yard area, there was an old tanker truck bed for the storage of corn/sugar syrup for feeding bees. There was also a small pond and number of new tree plantings with sawdust piled around the bases. Decomposing dead bees were noted in the yard area, on some of the sawdust piles and near the water source. Dead bee numbers were not excessive given the number of hives in the yard area. The piles of dead bees near some of the stacks of hives could be explained by normal mortality and/or losses from hive transport, especially if the number

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of hives in each stack was greater, as reported by Mark W. Some pesticide damage cannot be ruled out, but there was no evidence of significant losses.

Four hives were carefully inspected and sampled for bees, pollen, and fresh wax and honey, if present. Colony condition was good in 3 of the hives and moderate in one. Evidence of low-level virus infections in adults was detected in 3 hives and one hive had a few cells of chalkbrood. Entombed pollen was noted in one of the hives and samples were collected for analysis. None of the hives exhibited clear symptoms of pesticide damage. All hives had evidence of Varroa mite treatments.

The site contained 28 single-story hives (7 pallets of 4 hives) placed in an open location surrounded by recently planted cornfields. A small number of 'twitching' bees was observed in front of one of the hives but there were no large masses of dead or dying bees in front of any of the hives. Two hives were inspected and sampled, including the one with the dying bees in front. Bee, pollen and honey samples were collected from both hives, but a wax sample was collected from only one hive. One of the hives was in good condition, but the other was heavily infected with European foulbrood (EFB). Three other hives were inspected to determine general condition and all 3 exhibited problems. One had chalkbrood, one EFB, and the third was a non-functional laying worker colony. The beekeeper had indicated that this yard contained a number of poor colonies, but the problems appeared to more management related than pesticide related.

The Transformer site contained 32 single story hives (8 pallets of 4 hives) in a shaded apiary site surrounded by large trees. Two hives were carefully inspected and sampled for bees, pollen, and honey. Wax was collected from one hive. Both hives were in good condition and should be productive. Four other hives were examined; two had chalkbrood, one was queenless, and the other was in good condition. There was no evidence of pesticide damage in the bee yard and no indication from hive inspections of colony pesticide damage.

The apiary consisted of 36 two-story hives (on 9 pallets) set in a clearing in a wooded area. The hives were supered for honey production and were generally in good condition. Two hives were carefully inspected and sampled for bees, pollen, and honey. Both hives contained evidence of low-level virus infections (black shiny bees) and one colony had some chalkbrood. Both colonies had good queens as evidenced by solid brood patterns and were in good condition. Seven other hives were examined for general colony condition. Of the 4 hives that I inspected, one had chalkbrood, but the other 3 appeared healthy and in good condition. None of the hives at this site exhibited any signs of pesticide damage and there were no signs of a pesticide kill in the yard.

The was located in a cleared area at the edge of a woodlot and across the street from cornfields. The apiary contained 36 single-story hives (9 pallets of 4 hives). Two hives were examined carefully and sampled for bees, pollen, honey and wax, and two other hives were inspected for general hive condition.

Entombed pollen cells were found in 3 of the hives, indicating possible pesticide residues in some of the stored pollen. The two hives that were sampled appeared to be in reasonably good condition and showed no signs of pesticide damage. The other two inspected hives were in poorer condition; both were weak and exhibited signs of brood disease. One had chalkbrood and the other high levels of EFB. The second hive also had a poor queen as evidenced by a spotty brood pattern. There was no evidence of any pesticide kill in the bee yard.

Appendix B. Planting data for fields near staging area. All identified planters are pneumatic/vacuum type. Green indicates fields planted on or before May 14. Blue indicates Cruiser seed treatment (thiamethoxam). Red indicates Poncho seed treatment (Clothianidin). Orange indicates unknown seed treatment

Poncho seed treatment (Clothianidin). Orange indicates unknown seed treatment										
Field Name	Date Planted	Planter make	Hybrids and seed treatment	Seed treatment	Crop	Lubricant				
Corn field 1	no data	no data	no data	no data	no data	no data				
Corn field 2	no data	no data	no data	no data	no data	no data				
Corn field 3	no data	no data	no data	no data	no data	no data				
Corn field 4	no data	no data	no data	no data	no data	no data				
Corn field 5	no data	no data	no data	no data	no data	no data				
Corn field 6	no data	no data	no data	no data	no data	no data				
Corn field 7	5/10/2013	no data	Dekalb	unknown	Field corn	Talc				
Corn field 8	5/10/2013	no data	Dekalb	unknown	Field corn	Talc				
Corn field 9	5/9/2013	John Deere	Dekalb	Poncho	Field corn	Talc				
Corn field 10	5/7/2013	Case IH	Dekalb	unknown	Field corn	Graphite				
Corn field 11	5/7/2013	Case IH	Dekalb	unknown	Field corn	Graphite				
Corn field 12	no data	no data	no data	no data	no data	no data				
Corn field 13	no data	no data	no data	no data	no data	no data				
Corn field 14	no data	no data	no data	no data	no data	no data				
Corn field 15	6/3/2013	no data	Seed provided by Seneca Foods, hybrid unknown.	unknown	Sweet corn	none Talc/Graphite				
Corn field 16	5/8/2013	no data	Dekalb	Cruiser	Field corn	(eFlow)				
Corn field 17	5/8/2013	no data	Dekalb	Cruiser	Field corn	Talc/Graphite (eFlow)				
Corn field 18	5/8/2013	no data	Dekalb	Cruiser	Field corn	no data				
Corn field 19	5/11/2013	John Deere	Gold Country/ Wensman	Poncho	Field corn	Talc				
Corn field 20	5/13/2013	John Deere	Dekalb	none	Field corn	no data				
Corn field 21	5/16/2013	no data	Golden Harvest.	Cruiser	Field corn	Talc/Graphite (eFlow)				
Corn field 22	5/11/2013	John Deere	Gold Country/ Wensman	Poncho	Field corn	Talc				
Corn field 23	5/11/2013	John Deere	Gold Country/ Wensman	Poncho	Field corn	Talc				
Corn field 24	5/11/2013	John Deere	Gold Country/ Wensman	Poncho	Field corn	Talc				
Corn field 25	5/11/2013	John Deere	Gold Country/ Wensman	Poncho	Field corn	Talc				

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Corn field 26	5/16/2013	no data	Golden Harvest	Cruiser	Field corn	Talc/Graphite (eFlow)
Corn field 27	5/8/2013	no data	Dekalb	Cruiser	Field corn	Talc/Graphite (eFlow)
Corn field 28	5/8/2013	no data	Dekalb	Cruiser	Field corn	Talc/Graphite (eFlow)
Corn field 29	5/8/2013	no data	Dekalb	Cruiser	Field corn	Talc/Graphite (eFlow)

Appendix C. Planting data for fields near Gaylord transfer area. All identified planters are pneumatic/vacuum type. Green indicates fields planted on or before May 14. Blue indicates Cruiser seed treatment (Thiamethoxam). Red indicates Poncho seed treatment (Clothianidin). Purple cells indicate both Cruiser and Poncho. Orange indicates unknown seed treatment

Field	Dete	Diameter		Seed treatment		
Field Name	Date Planted	Plante r make	Hybrids		Сгор	Lubricant
Corn Field	5/10/2013	Case	dekalb 5378 Dekalb 5066 Dekalb 5262.	Poncho 500 Poncho 250 Poncho 250	Field Corn	Graphite
Corn Field 2	No data	No data	No Data	No Data	No Data	No Data
Corn Field 3	5/10/2013	Case IH	Dekalb 5356. Channel 20344	Poncho 500	Field Corn	Graphite
Corn Field 4	No Data	No Data	No Data	No Data	No Data	No Data
Corn Field 5	5/8/2013	John Deere	Dekalb 5356	Poncho/Votivo	Field Corn	Talc/Graphite (eFlow)
Corn Field 6	5/10/2013	Case IH	Pioneer 0062.	Cruiser	Field Corn	Graphite
Corn Field 7	No Data	No Data	No Data	No Data	No Data	No Data
Corn Field 8	5/6/2013	Case IH	Pioneer 0062.	Cruiser	Field Corn	Graphite
Corn Field 9	5/14/2013	White	Dekalb 5378. Dekalb 5066.	Poncho 250 Poncho 250	Field Corn	none
Corn Field	5/8/2013	Case IH	Dekalb 5378. GH -77774 3000 GT PI 0297.	Poncho 500 Cruiser 250	Field Corn	Graphite
Corn Field 11	5/16/2013	White	Dekalb 5066. Poncho 250	Poncho 250	Field Corn	none
Corn Field 12	No Data	Case IH	Dekalb. Poncho Trelay Poncho	Poncho	Field Corn	Graphite
Corn field 13	5/14/2013	Case IH	Trellay 7ST339RIB. Poncho Dekalb 5066. Poncho 250	Poncho	Field Corn	Graphite
Corn field 15	5/15/2013	Case IH	Dekalb 5066	Unknown	Field Corn	Graphite

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Corn field	5/10/2013	John Deere	Pioneer 0062. AcreMaxXtra Pioneer 0392. AcreMaxRw	Unknown	Field Corn	Talc
Corn Field 17	5/13/2013	White	Pioneer	Poncho	Field Corn	Talc
Corn Field 18	5/13/2013	No Data	Pioneer AcreMaxXtra. Pioneer AcreMaxRw.	Poncho	Field Corn	Talc
Corn Field 19	5/10/2013	John Deere	Pioneer 0062. AcreMaxXtra Pioneer 0392. AcreMaxRw	Unknown	Field Corn	Talc
Corn Field	5/8- 5/9/2013	Case IH	Dekalb 5378. Dekalb 4812. Pioneer 9526 AMX.	Poncho 500 Poncho 500 Poncho 1250	Field Corn	Graphite

Final Report

Investigation of a May 15, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticidetreated Maize Seed near Penfield, IL

Report Number

-130516

1025208-001 2025208-001 2025484-004

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

2 T.W. Alexander Drive

Research Triangle Park, North Carolina 27709

1.0 Background

On May 16, 2013, Mark O'Rourke of Bayer CropScience (BCS) received an email from a beekeeper who resides in Penfield, Illinois, notifying BCS of increased numbers of dead bees in front of several of hives at her farm. During a May 17, 2013 visit to the apiary indicated that she had observed large numbers of dead bees on the ground in front of all five of the hives in her apiary. She said that the onset of the observations occurred on May 15, the day following corn planting in fields adjacent to the apiary. The agreed that increased bee mortality at the hives continued for three days following planting.

The agreed to allow BCS personnel access to her apiary to investigate the incident.

2.0 Investigative Actions

2.1 Field Methods

Mark O'Rourke visited the site on May 17 and collected one sample of freshly dead and dying bees in front of one of the five affected hives. The area had been freshly swept the day before, resulting in samples that did not include the dead bees reported on May 16. Corn planting in adjacent fields was observed during the investigation.

Upon collection, the sample was placed in a field cooler, transferred to a laboratory freezer and shipped frozen via overnight express courier service to the BCS Residue Analysis Lab in Research Triangle Park, North Carolina.

At her request, Mr. O'Rourke did not contact the grower who had planted the corn field next to apiary. She did not provide names of area farmers.

Pertinent daily weather data (temperature, humidity, rainfall, daily wind conditions, etc.) recorded at a weather station 4.5 miles southeast of the bee yard were obtained from wunderground.com.

2.2 Laboratory Analysis

The dead bee sample was received frozen by the BCS Analytical group in Research Triangle Park, North Carolina and kept frozen until analysis. To prevent cross contamination, the sample was weighed into an individual centrifuge tube containing zircon beads. The sample was extracted with a modified QUECHERS method using a MiniLys bead mixer. The sample extract was analyzed by high resolution LC/MS-MS, with quantitation against isotopic internal standards added to the extract.

The analytical method quantified the levels of clothianidin, TZNG (a degradate of clothianidin) and thiamethoxam. Clothianidin and thiamethoxam are the main insecticidal active ingredients applied as corn seed treatments. In addition, samples were screened for presence of several other insecticide and miticide active ingredients including imidacloprid, carbaryl, chlorpyrifos, atrazine, fluvalinate, coumaphos, amitraz, and several degradates of these.

3.0 Results and Discussion

3.1 Weather Conditions Prior to and During the Incident

Records from a nearby weather station in Hope, II show wind blowing from the southwest throughout the day on May 15. Wind speed was 15-20 mph with gusts up to 28 mph during the late-morning hours. The apiary was downwind of planted fields on May 15 (Figure 3). The temperature on May 15 ranged from a low of 67.0 °F to a high of 88.0 °F. No rainfall occurred in the three days preceding or following the first observation of elevated mortality on May 15 (Figure 1).

3.2 Results of Survey of Surrounding Landscape

Hives are in close proximity to both CRP vegetation and fields immediately adjacent to apiary (Figure 2 and 3). Moderate coverage of seasonally blooming forage, including dandelions, in the adjacent CRP land was the only natural flowers available at the time (Figure 2). Planting data for adjacent fields is unknown.

3.3 Observations of Bee Mortality and Behavioral Impairment

Dead bees were observed by on May 15 and Mr. O'Rourke on May 17 at all five hives in the apiary. Mr. O'Rourke estimated that the number of dead bees at the hives entrances ranged from 50-100 bees and some elevated mortality was observed for three days (Figure 4). No behaviorally impaired bees were observed.

3.4 Pesticide Residue Analysis Results

Results of analysis of samples for pesticide residues are given in Table 1.

The dead bee sample collected by Mr. O'Rourke on May 17 contained 7.6 ng/g clothianidin, 2.3 ng/g TZNG (a metabolic break down product of clothianidin) and 3.7 ng/g thiamethoxam. The total neonicotinoid loads are in the range reported in previous incidents in which it was concluded that this level of exposure likely contributed to the elevated level of mortality that was observed. Clothianidin is itself a breakdown product of thiamethoxam and bees dosed with thiamethoxam may have mainly clothianidin residues in their bodies' post-mortem. The presence of thiamethoxam indicates the bees were likely exposed directly to this compound. It is unknown whether they were also exposed directly to clothianidin. This dead bee sample also was found to contain detectable residues of atrazine and carbaryl, However, the levels detected do not implicate these compounds as a likely cause of death.

The threshold lethal dose for clothianidin and thiamethoxam in honey bees is about 1 ng/bee and the LD_{50} dose for oral exposure is about 4 ng/bee. Since individual bees weigh approximately 100 mg, the theoretical concentration expected if a bee ingests a potentially lethal dose is >10 ng/g while a LD_{50} dose should produce a residue of about 40 ng/g. These calculations do not take into account any metabolism of degradation of the chemical occurring between the initial dosing and the measurement of residues. Past investigations of bee mortality incidents believed to be caused by exposure to clothianidin-laden dust have generally found residue levels to be greater than 5 ng/g in dead bees sampled from affected hives (Pistorius et al. 2009).

Investigations of honey bee incidents performed by the UK government compare measured residues of chemicals in dead bee samples to a subsequent residue level (SRL). These SRL's are determined by measuring the residues of bees dosed at the level of the LD50 in the laboratory. SRL's reported by Grieg-Smith et al. (1994) range from 1.7 to 20% of the applied dose. Laurino et al. (2011) dosed honey bees

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with clothianidin at several levels and then measured the resultant residues. The lowest dose tested, 3.28 ng/bee resulted in 87% mortality at 48 hr and a clothianidin residue of 0.8 ng/bee. This residue is 24% of the administered dose and is similar to the range reported by Grieg-Smith (1994). Assuming a honey bee body mass of 0.128 g, this results in a concentration of 6.25 ng/g.

The LD50 and SRL for carbaryl is 1300 and 70 µg/bee (Grieg-Smith et al. 1994). Using the assumption of a 0.128 g honey bee body mass, the SRL would be 547 ng/g carbaryl.

3.5 General Health Status of the Hives Involved

No quantitative inspection of the hives was made during the investigation. estimated that the impact on the colony health was minimal and that there would be no long-term effects on colony health and strength.

4.0 Discussion

Based on the magnitude of residues of clothianidin and thiamethoxam in the dead bee sample collected by O'Rourke, it is likely that exposure to a neonicotinoid (either thiamethoxam alone, or a combined exposure to both thiamethoxam and clothianidin) contributed to the observed elevated bee mortality. The timing and location of the incident suggests that the route of exposure may have been from dust emitted during the planting of corn.

The observed level of bee mortality, while clearly undesirable, did not appear to pose a serious risk of colony loss for the affected colonies. Colonies appear strong enough to recover to normal strength within a few weeks.

5.0 References

Grieg-Smith P.W., Thompson H.M., Hardy A.R., Bew M.H., Findlay E., Stevenson J.H. 1994. Incidents of poisoning of honeybees (Apis mellifera) by agricultural pesticides in Great Britain 1981-1991. Crop Protection 13(8):567-581.

Pistorius J., Bischoff G., Heimbach U., Stähler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Küln-Archiv 423:118-126.

Laurino D., Porporato M., Patetta A., Manino A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. Bulletin of Insectology 64(1):107-113.

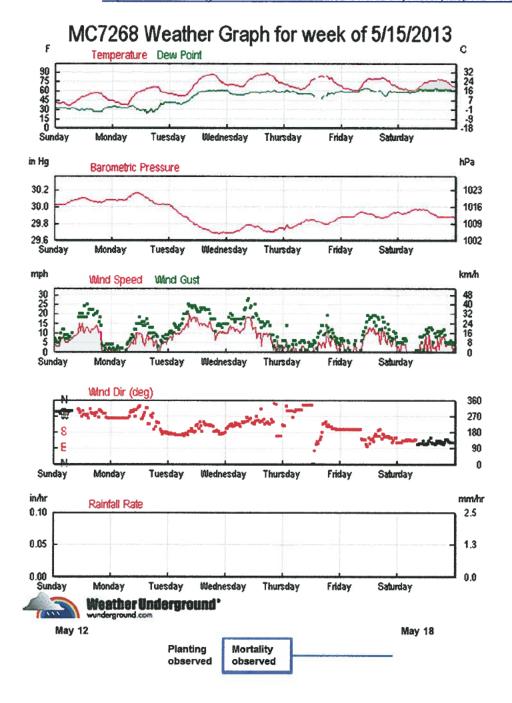
Table 1. Analytical chemistry results.

i	Sample	Date	Sample type	Concentration in ng/g					
	ID	Collected		Clothianidin	TZNG	Thiamethoxam	Other findings		
							Atrazine 18.0		
	IL-001	17-May	dead bees	7.6	2.3	3.7	Carbaryl 9.3		

Limit of detection = 0.5 ng/g for clothianidin, 0.4 ng/g for TZNG (a metabolite of clothianidin) and 0.6 ng/g for thiamethoxam, 1.3 ng/g for Atrazine, and 1.5 ng/g for Carbaryl.

Figure 1. Weather History for Hines, IL for May 12, 2013 through May 18, 2013.

Data Source: http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=MC7268



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Figure 2. apiary (top) and surrounding environment (bottom)





Figure 3. Location of apiary (blue marker) and surrounding landscape

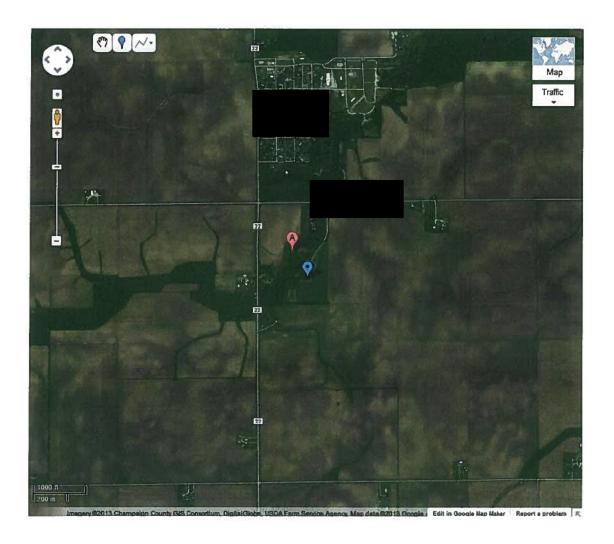


Figure 4. Dead bees in front of hive prior to collection for testing



Appendix 1. report to the EPA

On Wednesday, May 22, 2013, a beekill incident report was received through <u>beekill@epa.gov</u> (see Attachment). In a follow-up discussion with the beekeeper on May 23, 2013, the following information was obtained. The beekeeper has maintained 20 – 25 colonies since 1976 and describes themself as a hobbyist. The colonies are in groups of 5 and are located in several locations in the vicinity of Penfield, Illinois. Last year the beekeeper had noticed dead bees around 5 of her colonies located on their 55-acre farm at roughly the time when corn was being planted in fields located within 3 – 4 miles of their location. These colonies are positioned on corrugated tin which makes it easier to notice dead/dying bees within 5 feet of the colonies. Because their loss last year, the beekeeper was interested in whether a similar loss would occur this year.

Corn planting was early (April) last year; however, this year, the planting has been delayed by moist conditions in the surrounding fields. On the day they started planting corn in the area, the beekeeper immediately started to see dead bees around her colonies. On Tuesday, May 14, there were not many dead bees, but on May 15 there were more. They contacted the local field representative of Bayer CropScience who agreed to collect samples of bees for residue analyses. Bayer collected fresh samples on Friday, May 17.

The beekeeper's farm is partially in Conservation Reserve Program (17 acres) of which approximately one third is in the Natural Resource Conservation Service's Pollinator Enhancement Program and is seeded with pollinator attractive plants that are not yet in bloom. The colonies are position by wooded areas so that bees can forage on a diverse variety of plants. Fallow corn fields in the area are covered by a yellow-flowering weed Cressleaf Groundsel (Senecio glabellus; considered poisonous to grazing animals) and growers in the area "burn-down" the weed with herbicides prior to seeding corn. The weeds are attractive go bees especially given the low number of blooming plants at this time.

All of the affected colonies have remained queen right; however, the beekeeper noted that both older forage bees as well as younger hive bees (nurse bees) appear to be affected. The beekeeper believes that all of their colonies have been affected; however, the number of dead bees in these other yards is difficult to enumerate since the colonies are on grass.

Weather conditions last week while the planting was going on and the beekill incident was underway, were dry and windy with winds blowing out of the south.

The affected colonies are full size with approximately 45,000 bees/colony. The beekeeper describes 2 of the colonies as being in good condition, 2 in moderate condition, and one in poor condition. The colonies in good condition were recently split to prevent swarming.

The beekeeper relies on drone cell removal as a means of controlling varroa mite and they have not had to treat with flumigillin to control Nosema. They do not typically feed their colonies, but instead leave

sufficient honey to promote overwintering. While they may use pollen supplement, they try to avoid this since it can attract hive beetle.

Their colonies are registered. The beekeeper has collected samples for dead/dying bees and has placed them in the freezer if needed for residue analyses. The beekeeper has discussed her loss with the Illinois Dept. of Agriculture. The beekeeper is not claiming that the incident is a result of the planting of corn; however, she noted that it was coincident with the planting of corn in their vicinity. Although a similar event occurred last year, the affected colonies recovered and went on to produce a Fall honey crop.

According to the beekeeper who is president of a local beekeeping organization, several other beekeepers in their organization have experienced similar losses. However, one of the beekeepers in her organization has experienced "high" losses and attributes those losses to the planting of corn immediately adjacent to his colonies. This other beekeeper has reported his losses to the Illinois Department of Agriculture.

The beekeeper is willing to relay the results of the residue analyses to EPA once those data are available.

Report submitted by

Attachment

From:

To:

Beekill@EPA

Date:

05/23/2013 08:57 AM

Subject: RE: FW: tumbler bees

Thank you for responding! I actually sent you two messages-one that I sent to Randy Oliver (who recommended that I contact EPA) and one that I sent to a nearby representative of Bayer Crop Sciences which had my phone number on it. I would prefer to speak with you instead of email. My cell phone # is

I will try to be available after 10:00 this morning. I had a long conversation with someone who called me from theIll. Dept. of Ag yesterday (another beekeeper referenced me).

From: Steeger.Thomas@epamail.epa.gov [Steeger.Thomas@epamail.epa.gov] on behalf of beekill@epa.gov [beekill@epa.gov] Sent: Thursday, May 23, 2013 6:08 AM To:

Page 11 of 14

Cc: steeger.thomas@epa.gov
Subject: Re: FW: tumbler bees

Dear

Thank you for contacting the U.S. Environmental Protection Agency through beekill@epa.gov regarding your recent loss of bees. I would like to learn more about the circumstances surrounding this loss. If you provide a contact number, I would be willing to call you; alternatively, I can provide some written questions to which you could respond via email. Please let me know your preference. Also, I will be interested in whether you contacted the state of Illinois regarding this loss and whether they have investigated the incident.

I see that you are associated with Department of Entomology at the University of Illinois. My colleagues and I have benefited from the input that other members of your department have been able to provide on factors associated with bee losses. I hope that you will afford me the opportunity to speak with you regarding your recent loss of bees.

Due to sequestration, all EPA offices will be closed on Friday, May 24 in advance of the Memorial Day weekend. I apologize in advance if you attempt to contact me during this time and it appears as though I am not responsive.

Sincerely,

Tom Steeger Thomas Steeger, Ph.D. Senior Science Advisor Environmental Fate and Effects Division U.S. EPA Office of Pesticide Programs (703)305-5444 (office) steeger.thomas@epa.gov

[Inactive hide details for ---05/22/2013 09:17:48 AM---FYI: this was the info that I sent to Randy Oliver abo] ---05/22/2013 09:17:48 AM---FYI: this was the info that I sent to Randy Oliver about my beekill. I will also forward the communi

From:

To:

Page 12 of 14

Beekill@EPA

Date:

05/22/2013 09:17 AM

Subject:

FW: tumbler bees

FYI: this was the info that I sent to Randy Oliver about my beekill. I will also forward the communication I sent to our nearest Bayer representative who was quick to respond and collect samples on site. Probably too little too late on my part...not as bad as last year

From:
Sent: Thursday, May 16, 2013 7:37 AM
To: randy@randyoliver.com
Subject: tumbler bees

The window for planting corn opened on Tuesday here in east central Illinois. I was working on a 5 colony yard near my home and saw a few dead and dying bees on the corrugated tin which the colonies sit upon. Yesterday (Wed) there were more, so I gathered up my soft forceps, rubbing alcohol for sterilizing the forceps, ziplock baggies and latex gloves and carefully collected two 20 bees samples (dying foragers-appeared young and fuzzy), and put them immediately into the freezer. I posted seeing this last year on Beesource, wondering if it was just my observation, and was contacted by the local Bayer rep, who offered to test samples (collected in this manner) should I see this again.

Although the hour was late, I collected a smaller sample from another yard in the same manner.

Randy, you may remember this conversation from last spring about this...I am the retired field lab manager from Gene Robinsons beelab at UI. Would you be interested in a sample? I intend to check again today and collect if the same is

Not exactly tumblers, but pathetic young foragers neverthless.

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 $(See\ attached\ file:\ graycol.gif) (See\ attached\ file:\ ecblank.gif)$

Final Report

Investigation of a May 20, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticidetreated Maize Seed near North Manchester, IN

Report Number

-130520

1025484-005

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

2 T.W. Alexander Drive

Research Triangle Park, North Carolina 27709

1.0 Background

On May 20, 2013 Dick Rogers of Bayer CropScience (BCS) was contacted by a commercial beekeeper located in Lafontaine, IN, about large numbers of dead bees in front of hives at one of his apiaries. On May 20, observed piles of 100-200 dead bees at the entrances of each of the 20 hives located at his apiary in North Manchester, IN. suspected corn planting in nearby fields as the cause of elevated hive mortality. The date of corn planting is uncertain because had not visited the apiary in the three weeks prior to his observation of dead bees. Mark O'Rourke (BCS) visited the apiary on May 21 and collected a sample for residue analysis.

2.0 Investigative Actions

2.1 Field Methods

Mark O'Rourke visited the site on May 21 and collected two samples of dead bees. Blooming forage was observed in field boundaries and ditches and in a nearby wooded area. Corn planting in adjacent fields was not observed during the investigation but occurred some time in the three weeks prior to May 20.

Upon collection, the samples were placed in a field cooler, transferred to a laboratory freezer and shipped frozen via overnight express courier service to the BCS Residue Analysis Lab in Research Triangle Park, North Carolina.

The grower who had planted the field adjacent to apiary was not contacted.

Pertinent daily weather data (temperature, humidity, rainfall, daily wind conditions, etc.) recorded at a weather station 19 miles north of the bee yard were obtained from wunderground.com.

2.2 Laboratory Analysis

Samples were received frozen by the BCS Analytical group in Research Triangle Park, North Carolina and kept frozen until analysis. To prevent cross contamination, all samples were weighed into individual centrifuge tubes containing zircon beads. The samples were extracted with a modified QUECHERS method using a MiniLys bead mixer. Sample extracts were analyzed by high resolution LC/MS-MS, with quantitation against isotopic internal standards added to each extract.

The analytical method quantified the levels of clothianidin, TZNG (a degradate of clothianidin) and thiamethoxam. Clothianidin and thiamethoxam are the main insecticidal active ingredients applied as corn seed treatments. In addition, samples were screened for presence of several other insecticide and miticide active ingredients including imidacloprid, carbaryl, chlorpyrifos, atrazine, fluvalinate, coumaphos, amitraz, and several degradates of these.

3.0 Results and Discussion

3.1 Weather Conditions Prior to and During the Incident

Records from a nearby weather station in North Manchester, IN for the week prior to May 20 show that no rainfall occurred in the week prior to May 20. Winds with gusts up to 30 mph occurred May 13-15 (Table 1, Figure 1).

Page 2 of 8

3.2 Results of Survey of Surrounding Landscape

The Fetters apiary is immediately adjacent to cornfields. Blooming forage was observed in field boundaries and ditches and in a nearby wooded area (Figure 2).

3.3 Observations of Bee Mortality and Behavioral Impairment

Dead bees were observed at all 20 hives in the apiary by on May 20 and O'Rourke on May 21. In the second Mr. O'Rourke observed 100-200 dead bees at each hive entrance. No behaviorally impaired bees were observed. Following an in-hive observation, concluded that the colonies were weak in comparison to hives at his other apiaries.

3.4 Pesticide Residue Analysis Results

Results of analysis of samples for pesticide residues are given in Table 2.

One of the dead bee samples collected by Mr. O'Rourke on May 21 contained 11.3 ng/g clothianidin and 4.4 ng/g TZNG, a clothianidin degradate. The total neonicotinoid load is in the range reported in previously incidents in which it was concluded that this level of exposure likely contributed to the elevated level of mortality that was observed The other dead bee sample collected by Mr. O'Rourke on May 21 contained only a trace amount of clothianidin, and no detectable TZNG. It is therefore unlikely that clothianidin exposure contributed significantly to the elevated mortality of this hive.

The threshold lethal dose for clothianidin and thiamethoxam in honey bees is about 1 ng/bee and the LD_{50} dose for oral exposure is about 4 ng/bee. Since individual bees weigh approximately 100 mg, the theoretical concentration expected if a bee ingests a potentially lethal dose is >10 ng/g while a LD_{50} dose should produce a residue of about 40 ng/g. These calculations do not take into account any metabolism of degradation of the chemical occurring between the initial dosing and the measurement of residues. Past investigations of bee mortality incidents believed to be caused by exposure to clothianidin-laden dust have generally found residue levels to be greater than 5 ng/g in dead bees sampled from affected hives (Pistorius et al. 2009).

Investigations of honey bee incidents performed by the UK government compare measured residues of chemicals in dead bee samples to a subsequent residue level (SRL). These SRL's are determined by measuring the residues of bees dosed at the level of the LD50 in the laboratory. SRL's reported by Grieg-Smith et al. (1994) range from 1.7 to 20% of the applied dose. Laurino et al. (2011) dosed honey bees with clothianidin at several levels and then measured the resultant residues. The lowest dose tested, 3.28 ng/bee resulted in 87% mortality at 48 hr and a clothianidin residue of 0.8 ng/bee. This residue is 24% of the administered dose and is similar to the range reported by Grieg-Smith (1994). Assuming a honey bee body mass of 0.128 g, this results in a concentration of 6.25 ng/g.

The LD50 and SRL for carbaryl is 1300 and 70 μ g/bee (Grieg-Smith et al. 1994). Using the assumption of a 0.128 g honey bee body mass, the SRL would be 547 ng/g carbaryl.

3.5 General Health Status of the Hives Involved

No quantitative inspection of the hives was made during the investigation. However, following an in-hive observation concluded that the colonies were weak in comparison to similar hives at his other apiaries indicated that time and hive management would be required to re-build the strength of the colonies.

4.0 Discussion

While it is clear that corn was planted in the vicinity of the apiary where the elevated mortality occurred, it is unknown if the mortality event was coincident with planting of a nearby cornfield since the exact dates of each are unknown. Results of residue analysis of dead bee samples are inconsistent. One sample had levels of clothianidin that suggest clothianidin contributed to the observed mortality, the other had levels suggesting it did not. Based on available evidence, it can be concluded that clothianidin likely contributed to the observed mortality at some of the hives in this apiary, but not all of them.

5.0 References

Grieg-Smith P.W., Thompson H.M., Hardy A.R., Bew M.H., Findlay E., Stevenson J.H. 1994. Incidents of poisoning of honeybees (Apis mellifera) by agricultural pesticides in Great Britain 1981-1991. Crop Protection 13(8):567-581.

Pistorius J., Bischoff G., Heimbach U., Stähler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Küln-Archiv 423:118-126.

Laurino D., Porporato M., Patetta A., Manino A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. Bulletin of Insectology 64(1):107-113.

Table 1. Weather history for May 1 to May 21, 2013 in Warsaw, IN

Source: http://www.wunderground.com/history/airport/KASW/

2013	Temp. (°F)			Ht	Humidity (%)		Wind (mph)			Precip. (in)	Events
May	high	avg	low	high	avg	low	high	avg	high	sum	
1	80	66	51	94	58	39	12	3	20	0	-
2	78	68	59	82	61	41	13	5	20	0	
<u>3</u>	75	63	51	94	65	44	17	5	22	0	
4	71	64	57	59	47	27	16	9	25	0	
<u>5</u>	69	60	50	76	57	43	16	8	24	0	Rain
<u>6</u>	71	60	50	71	55	42	10	6	18	0	Rain
7	73	62	50	94	69	43	13	3	18	0	
<u>8</u>	75	62	50	100	70	41	17	1	-	0.02	Rain
9	78	63	48	100	70	34	12	2	17	0.02	Rain
<u>10</u>	64	54	44	100	86	68	17	9	22	0.34	Rain
<u>11</u>	55	46	35	100	84	57	22	8	30	0.1	Rain
<u>12</u>	50	41	32	93	67	43	23	9	28	0	
<u>13</u>	59	46	33	100	56	23	15	3	22	0	
<u>14</u>	84	66	48	61	45	28	16	5	25	0	Rain
<u>15</u>	82	73	64	68	49	32	20	10	31	0	Rain
<u>16</u>	80	65	50	100	54	21	6	1	-	0	
<u>17</u>	78	66	53	88	58	36	12	4	21	0	
<u>18</u>	77	66	55	73	56	47	10	6	16	0	
<u>19</u>	86	70	55	77	61	43	13	4	16	0	
<u>20</u>	84	76	69	94	68	51	16	5	24	0	
<u>21</u>	82	72	62	100	68	42	22	7	32	0.1	Rain

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Table 2. Analytical chemistry results.

	Date			Conce	ntration in ng/g (p	pb)
Sample ID	Collected	Sample type	Clothianidin	TZNG	Thiamethoxam	Other findings
						Amitraz 110
E.						Imidacloprid <lod< td=""></lod<>
-IN-		Bees				Atrazine 23
001	23-May	004	0.6	<lod< td=""><td><lod< td=""><td>Carbaryl 6</td></lod<></td></lod<>	<lod< td=""><td>Carbaryl 6</td></lod<>	Carbaryl 6
						Amitraz 10
						Imidacloprid <lod< td=""></lod<>
IN		Page				Atrazine 55
-IN- 002	23-May	Bees 005	11.3	4.4	<lod< td=""><td>Carbaryl 3</td></lod<>	Carbaryl 3

Limit of detection = 0.5 ng/g for clothianidin, 0.4 ng/g for TZNG (a metabolite of clothianidin) and 0.6 ng/g for thiamethoxam, 1.3 ng/g for Atrazine, and 1.5 ng/g for Carbaryl.

Figure 1. Weather History for May 1 to May 21, 2013

Source: http://www.wunderground.com/history/airport

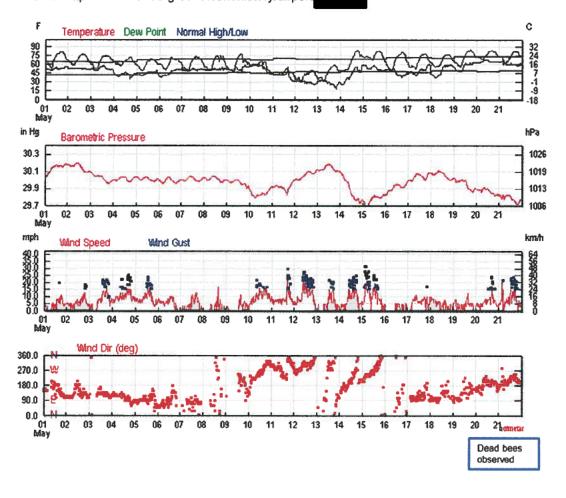
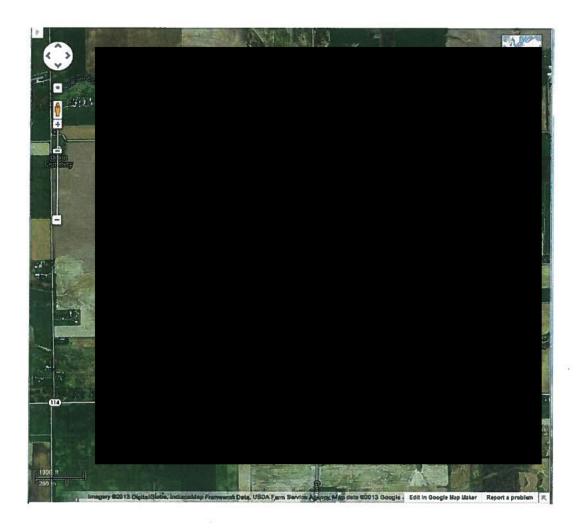


Figure 2. apiary location and surrounding area.



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Final Report

Investigation of a May 16, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticide-treated Maize Seed near Champaign, IL

1025187-001 1025290-001 1025484-006

Report Numbers

130520

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

2 T.W. Alexander Drive

Research Triangle Park, North Carolina 27709

1.0 Background

On May 21, 2013, a Bayer CropScience (BCS) representative contacted who resides in Rantoul, Illinois, by phone concerning reports of large numbers of dead	
total of 8 hives located among three apiaries.	
his Home yard containing five hives beginning on May 16 2013, and first observed dea	
that site May 17 2013. Can also an analysis and and owner, the site of one hive, observed planting	ng in an
adjacent field on May 14 and 15 and observed dead bees May 16.	
site of two recently created hives, reported planting May 16 and May 17 and first obse	rved dead
bees on May 18. reported that increased bee mortality at the hives continued	d for three
to four days following planting. agreed to allow BCS personnel access to his	apiary to
investigate the incident reported the incident to the EPA and the Illinois Dep	artment of
Agriculture on May 17. Prior to the BCS investigation the Illinois Department of Agricul	ture bee
inspector, Rick Davis, collected all recently dead bees from each hive location.	reported
that Mr. Davis also collected hive surface wipe samples from each location. The incide	ent was
reported to the EPA by	

2.0 Investigative Actions

2.1 Field Methods

Mark O'Rourke visited the site on May 22 and collected two samples of freshly dead or dying bees from separate hives at Home and Applications application (Figure 1). In maintains a total of eight hives at his three applications. Dead bees had been freshly collected the day before by IL Dept. of Ag. inspector Rick Davis.

Upon collection, the sample was placed in a field cooler, transferred to a laboratory freezer and shipped frozen via overnight express courier service to the BCS Residue Analysis Lab in Research Triangle Park, North Carolina.

Local growers were not contacted to obtain planting records.

Pertinent daily weather data (temperature, humidity, rainfall, daily wind conditions, etc.) recorded at the Rantoul, IL weather station located nome, and Hawks bee yards, respectively, were obtained from wunderground.com.

2.2 Laboratory Analysis

Samples were received frozen by the BCS Analytical group in Research Triangle Park, North Carolina and kept frozen until analysis. To prevent cross contamination, all samples were weighed into individual centrifuge tubes containing zircon beads. The samples were extracted with a modified QUECHERS method using a MiniLys bead mixer. Sample extracts were analyzed by high resolution LC/MS-MS, with quantitation against isotopic internal standards added to each extract.

The analytical method quantified the levels of clothianidin, TZNG (a degradate of clothianidin) and thiamethoxam. Clothianidin and thiamethoxam are the main insecticidal active ingredients applied as corn seed treatments. In addition, samples were screened for presence of several

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other insecticide and miticide active ingredients including imidacloprid, carbaryl, chlorpyrifos, atrazine, fluvalinate, coumaphos, amitraz, and several degradates of these.

3.0 Results and Discussion

3.1 Weather Conditions Prior to and During the Incident

Weather conditions for May 12 to May 25, 2013 are given in Table 1 and Figure 2.
On May 14, the first day of observed planting at the winds were blowing 10-20 mph from the SSW with gusts up to 25 mph. The temperature on May 14 ranged from a low of 54 °F to a high of 89 °F. Humidity ranged from 33% to 69%.
On May 15, the second day of observed planting at the winds were blowing 10-2 mph from the WSW with gusts up to 30 mph. The temperature on May 15 ranged from a low of 70 °F to a high of 90 °F. Humidity ranged from 30% to 62%.
On May 16, the first day of observed elevated mortality at the planting near the winds were blowing 0-10 mph from the WNW in the morning, shifting to a blowing from the south in the mid-day. The temperature on May 16 ranged from a low of 65 °F to a high of 84 °F. Humidity ranged from 25% to 78%. 0.33 inches of rainfall on May 16 was the only rainfall that occurred in the three days preceding or following the first observation of elevated mortality on May 16 (Figure 2).
On May 17, the first day of observed elevated mortality at the and the second day of planting near the winds were blowing 5-15 mph from the SSE with gusts up to 20 mph. The temperature on May 17 ranged from a low of 62°F to a high of 83°F. Humidity ranged from 31% to 91%
On May 18, the first day of observed elevated mortality at the winds were blowing 0-10 mph from the ESE. The temperature on May 18 ranged from a low of 61°F to a high of 82°F. Humidity ranged from 45% to 89%.
3.2 Results of Survey of Surrounding Landscape
Hives are in close proximity to blooming vegetation and fields adjacent to all three apiaries (Figures 3 and 4). In addition, the Scudders yard is located on CRP land. Moderate coverage of seasonally blooming forage in an adjacent waterway, adjacent CRP land, and unplanted fields including dandelions and yellow rocket were the only natural flowers available at the time. Mr. O'Rourke observed corn planting in fields adjacent to all three apiaries during the investigation. The field east of the
3.3 Observations of Bee Mortality and Behavioral Impairment
Piles of dead bees were observed by on May 16 at the home apiary, May 16 at the apiary. O'Rourke observed old piles of dead bees on
May 22 at all five hives in the home apiary and the single hive at the part apiary. The yard had fewer dead bees in front of the NW hive than the other yards and what would be considered a normal number of dead bees in front of the SE hive. Elevated mortality was

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observed by for three to four days. Behaviorally impaired bees were observed by O'Rourke exhibiting twitching and staggering behavior.

3.4 Pesticide Residue Analysis Results

Results of analysis of samples for pesticide residues are given in Table 2.

The dead bee samples collected by Mr. O'Rourke on May 22 contained 10.6 and 4.4 ng/g, chlothianidin, respectively, indicating these bees had been exposed to clothianidin. The total neonicotinoid loads are in the range reported in previously incidents in which it was concluded that this level of exposure likely contributed to the elevated level of mortality that was observed.

The threshold lethal dose for clothianidin and thiamethoxam in honey bees is about 1 ng/bee and the LD $_{50}$ dose for oral exposure is about 4 ng/bee. Since individual bees weigh approximately 100 mg, the theoretical concentration expected if a bee ingests a potentially lethal dose is >10 ng/g while a LD $_{50}$ dose should produce a residue of about 40 ng/g. These calculations do not take into account any metabolism of degradation of the chemical occurring between the initial dosing and the measurement of residues. Past investigations of bee mortality incidents believed to be caused by exposure to clothianidin-laden dust have generally found residue levels to be greater than 5 ng/g in dead bees sampled from affected hives (Pistorius et al. 2009).

Investigations of honey bee incidents performed by the UK government compare measured residues of chemicals in dead bee samples to a subsequent residue level (SRL). These SRL's are determined by measuring the residues of bees dosed at the level of the LD50 in the laboratory. SRL's reported by Grieg-Smith et al. (1994) range from 1.7 to 20% of the applied dose. Laurino et al. (2011) dosed honey bees with clothianidin at several levels and then measured the resultant residues. The lowest dose tested, 3.28 ng/bee resulted in 87% mortality at 48 hr and a clothianidin residue of 0.8 ng/bee. This residue is 24% of the administered dose and is similar to the range reported by Grieg-Smith (1994). Assuming a honey bee body mass of 0.128 g, this results in a concentration of 6.25 ng/g.

The LD50 and SRL for carbaryl is 1300 and 70 μg/bee (Grieg-Smith et al. 1994). Using the assumption of a 0.128 g honey bee body mass, the SRL would be 547 ng/g carbaryl.

3.5 General Health Status of the Hives Involved

No quantitative inspection of the hives was made during the investigation. However, one of the two recently established hives at the Hawks apiary appeared to contain very few bees. Mr. Nuss estimated that the field force of the bees would recover within approximately three weeks.

4.0 Discussion

The circumstances reported suggest that bees may have been exposed to abraded corn seed dust. The analytical results confirm exposure to clothianidin, and this exposure contributed to the elevated mortality that was observed.

The observed level of bee mortality, while clearly undesirable, did not appear to pose a serious risk of colony loss for the affected colonies. Colonies appear strong enough to recover to normal strength within a few weeks.

5.0 References

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Grieg-Smith P.W., Thompson H.M., Hardy A.R., Bew M.H., Findlay E., Stevenson J.H. 1994. Incidents of poisoning of honeybees (Apis mellifera) by agricultural pesticides in Great Britain 1981-1991. Crop Protection 13(8):567-581.

Pistorius J., Bischoff G., Heimbach U., Stähler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Küln-Archiv 423:118-126.

Laurino D., Porporato M., Patetta A., Manino A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. Bulletin of Insectology 64(1):107-113.

Table 1. Weather History for Rantoul, IL for May 12, 2013 through May 25, 2013

Data Source: http://www.wunderground.com/history/airport/KTIP/

2013	Temp. (°F)		Wi	Wind (mph)			Events	
May	high	avg	low	high	avg	high	(in) sum	
<u>12</u>	59	48	38	17	10	28	0.00	
13	68	52	37	12	6	18	0.00	Rain
14	89	72	54	22	12	25	0.00	
<u>15</u>	90	80	70	22	14	30	0.00	Rain, Thunderstorm
<u>16</u>	84	74	65	12	6	20	0.00	Rain, Thunderstorm
17	83	72	62	15	5	20	0.00	Rain
18	82	72	61	12	4	-	0.00	
<u>19</u>	90	76	61	21	6	25	0.00	
<u>20</u>	89	80	72	28	14	34	0.00	Thunderstorm
<u>21</u>	82	73	64	22	14	30	0.33	Rain, Thunderstorm
<u>22</u>	77	70	62	21	13	32	0.11	Rain
<u>23</u>	62	54	45	20	11	28	0.01	Rain
24	68	54	39	15	10	18	0.00	
<u>25</u>	63	55	47	13	6	-	0.22	Rain

Table 2. Analytical chemistry results.

Sample	Date	Sample	Concentration in ng/g						
L ID	Collected	type	Clothianidin	TZNG	Thiamethoxam	Other findings			
Nuas	May 22	Dood				Imidacloprid 1.7 (only olefin)			
Nuss- IL-002	May 22, 2013	Dead	10.6	3.9	-I OD	Atrazine 1090			
IL-002	2013	bees	10.6	3.8	<lod< td=""><td>Carbaryl 2.0 Imidacloprid</td></lod<>	Carbaryl 2.0 Imidacloprid			
						1.1 (only olefin)			
Nuss-	May 22,	Dead				Atrazine 11			
IL-003	2013	bees	4.4	1.5	<lod< td=""><td>Carbaryl <lod< td=""></lod<></td></lod<>	Carbaryl <lod< td=""></lod<>			

Limit of detection = 0.5 ng/g for clothianidin, 0.4 ng/g for TZNG (a metabolite of clothianidin) and 0.6 ng/g for thiamethoxam, 1.3 ng/g for Atrazine, and 1.5 ng/g for Carbaryl.

Figure 1. Hives sampled for dead bees for testing



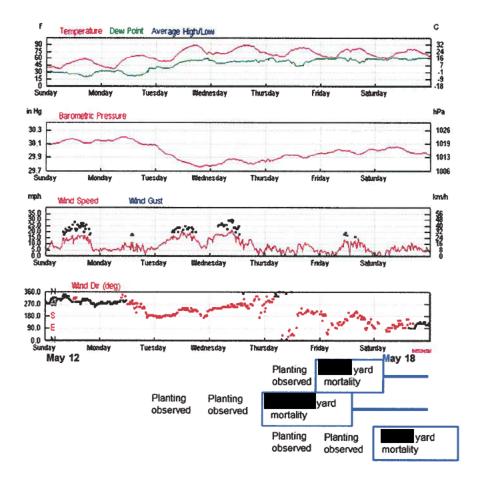
Apiary where sample Bees 002" was collected.

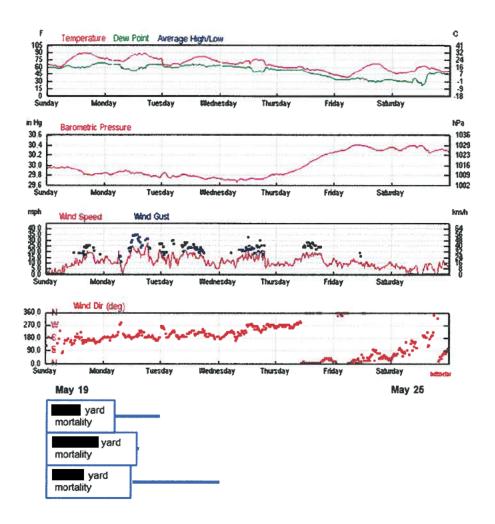


Hive where sample Bees 003" was collected.

Figure 2. Weather History for Rantoul, IL for May 12, 2013 through May 25, 2013.

Data Source: http://www.wunderground.com/history/airport/KTIP/





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Figure 3. apiaries and surrounding environment



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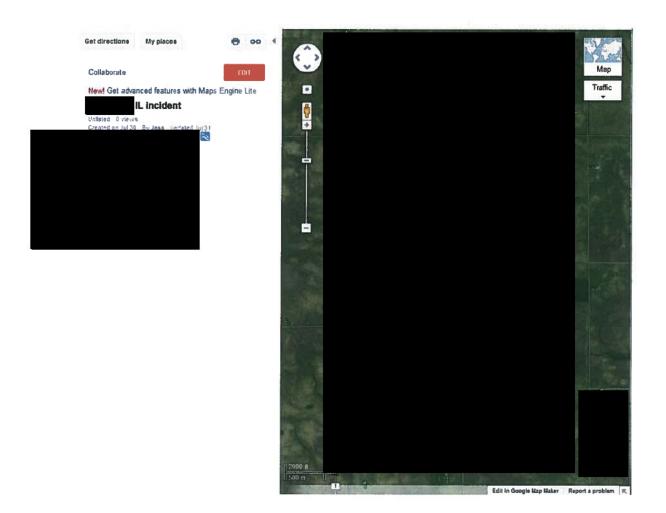




west edge looking West

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Figure 4. Apiary locations and surrounding area. Imagery reported on Google Maps as from 2013 Champaign County GIS Consortium.



Final Report

Investigation of a May 19, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticide-treated Maize Seed near Champaign, IL

update to 2025484-007

Report Number

130522

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

2 T.W. Alexander Drive

Research Triangle Park, North Carolina 27709

1.0 Background

On May 23, 2013 a BCS representative initiated contact with a beekeeper in Urbana, Illinois, regarding observations shared at the May 19 Central Eastern Illinois Beekeeping Association (CEIBA) meeting. observed dead and dying bees in front of her 14 hives at her home apiary on May 19 following corn planting she observed May 18 in adjacent fields. Mark O'Rourke, of BCS, visited apiary on May 23 to investigate the incident and collected two bee samples, one of dead and one of live bees. Bees were observed exhibiting unusual behavior, including clinging to vegetation in front of hive entrances.

2.0 Investigative Actions

2.1 Field Methods

Mark O'Rourke visited the site on May 23 and collected two samples of bees, one of dead bees and one sample of live behaviorally-impaired bees at 5:00 pm (Figure 1). Only one sample of dead bees was taken due to the low number of freshly dead bees. Blooming bee-attractive forage planted by was available in a yard adjacent to the apiary and planted fields. Corn planting in adjacent fields was observed during the investigation.

Upon collection, the sample was placed in a field cooler, transferred to a laboratory freezer and shipped frozen via overnight express courier service to the BCS Residue Analysis Lab in Research Triangle Park, North Carolina.

Mr. O'Rourke contacted the farmer who operates around appared apiary, to obtain planting information.

Pertinent daily weather data (temperature, humidity, rainfall, daily wind conditions, etc.) recorded at a weather station 1.5 miles south of the bee yard were obtained from wunderground.com.

2.2 Laboratory Analysis

Samples were received frozen by the BCS Analytical group in Research Triangle Park, North Carolina and kept frozen until analysis. To prevent cross contamination, all samples were weighed into individual centrifuge tubes containing zircon beads. The samples were extracted with a modified QUECHERS method using a MiniLys bead mixer. Sample extracts were analyzed by high resolution LC/MS-MS, with quantitation against isotopic internal standards added to each extract.

The analytical method quantified the levels of clothianidin, TZNG (a degradate of clothianidin) and thiamethoxam. Clothianidin and thiamethoxam are the main insecticidal active ingredients applied as corn seed treatments. In addition, samples were screened for presence of several other insecticide and miticide active ingredients including imidacloprid, carbaryl, chlorpyrifos, atrazine, fluvalinate, coumaphos, amitraz, and several degradates of these.

3.0 Results and Discussion

3.1 Weather Conditions Prior to and During the Incident

Records from a nearby weather station in show winds blowing 0-10 mph from the SE throughout the day on May 19, with gusts up to 19 mph. The temperature on May 19 ranged from a low of 60°F to a high of 88 °F. Humidity on May 19 ranged from a low of 49% to a high of 94%. No rainfall occurred in the three days prior to May 19. In the three days following May 19 0.53 inches of rainfall occurred on May 21, and 0.12 in. fell on May 22 (Figure 2).

hives would have been downwind of the field east of her apiary on May 19 (Figures 3 and 4).

3.2 Results of Survey of Surrounding Landscape

hives are in close proximity to both planted bee-attractive forage and fields immediately adjacent to the apiary (Figures 3 and 4). The farmer planted nearby cornfields using a 12-row John Deere 7000 series, finger pick-up planter, with three rows using Aztec (Tebupirimphos) insecticide (Amvac). The farmer used 1 oz of graphite per unit at each fill of the planter. The hybrids planted, DKC 62-08 RIB and DKC 65-17 RR (with Aztec), were all treated with Poncho (clothianidin). Aztec was added to the RR only hybrid as a second mode of action to control Corn Root worm as well as activity on the other secondary soil insects.

3.3 Observations of Bee Mortality and Behavioral Impairment

Dead bees were observed at all 14 hives in the apiary by on May 19 and Mr. O'Rourke on May 23. Mr. O'Rourke observed approximately 100 dead bees observed at each hive entrance and reported elevated mortality for four days. Behaviorally impaired bees were observed clinging to grasses in front of the hive entrance by and Mr. O'Rourke on May 23.

3.4 Pesticide Residue Analysis Results

Results of analysis of samples for pesticide residues are given in Table 1.

The dead bee sample collected by O'Rourke on May 23 contained 7.9 ng/g of clothianidin, 2.9 ng/g TZNG (a metabolic break down product of clothianidin) and 11 ng/g of atrazine. The total neonicotinoid loads are in the range reported in previously incidents in which it was concluded that this level of exposure likely contributed to the elevated level of mortality that was observed. The level of atrazine detected in the dead bee sample does not implicate the compound as a likely cause of death. Samples were not analyzed for the presence of Aztec, the insecticide added to the seeds planted in adjacent fields.

The live, behaviorally impaired, bee sample collected by O'Rourke on May 23 contained no detectable residues of any compounds in the residue analysis.

The threshold lethal dose for clothianidin and thiamethoxam in honey bees is about 1 ng/bee and the LD_{50} dose for oral exposure is about 4 ng/bee. Since individual bees weigh approximately 100 mg, the theoretical concentration expected if a bee ingests a potentially lethal dose is >10

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ng/g while a LD $_{50}$ dose should produce a residue of about 40 ng/g. These calculations do not take into account any metabolism of degradation of the chemical occurring between the initial dosing and the measurement of residues. Past investigations of bee mortality incidents believed to be caused by exposure to clothianidin-laden dust have generally found residue levels to be greater than 5 ng/g in dead bees sampled from affected hives (Pistorius et al. 2009).

Investigations of honey bee incidents performed by the UK government compare measured residues of chemicals in dead bee samples to a subsequent residue level (SRL). These SRL's are determined by measuring the residues of bees dosed at the level of the LD50 in the laboratory. SRL's reported by Grieg-Smith et al. (1994) range from 1.7 to 20% of the applied dose. Laurino et al. (2011) dosed honey bees with clothianidin at several levels and then measured the resultant residues. The lowest dose tested, 3.28 ng/bee resulted in 87% mortality at 48 hr and a clothianidin residue of 0.8 ng/bee. This residue is 24% of the administered dose and is similar to the range reported by Grieg-Smith (1994). Assuming a honey bee body mass of 0.128 g, this results in a concentration of 6.25 ng/g.

The LD50 and SRL for carbaryl is 1300 and 70 μ g/bee (Grieg-Smith et al. 1994). Using the assumption of a 0.128 g honey bee body mass, the SRL would be 547 ng/g carbaryl.

3.5 General Health Status of the Hives Involved

and Mr. O'Rourke estimated that the impact on the colony health was minimal and that there would be no long-term effects on colony health and strength.

4.0 Discussion

The occurrence of the observed bee mortality and the analytical results are consistent with exposure of bees to clothianidin, possibly in the form of abraded corn seed dust released during planting. The level of clothianidin in the dead bee sample confirms exposure to this compound likely contributed to the elevated mortality that was observed.

The observed level of bee mortality, while clearly undesirable, did not appear to pose a serious risk of colony loss for the affected colonies. Colonies appear strong enough to recover to normal strength within a few weeks.

5.0 References

Grieg-Smith P.W., Thompson H.M., Hardy A.R., Bew M.H., Findlay E., Stevenson J.H. 1994. Incidents of poisoning of honeybees (Apis mellifera) by agricultural pesticides in Great Britain 1981-1991. Crop Protection 13(8):567-581.

Pistorius J., Bischoff G., Heimbach U., Stähler M. 2009. Bee poisoning incidents in Germany in spring 2008 caused by abrasion of active substance from treated seeds during sowing of maize. Julius-Küln-Archiv 423:118-126.

Laurino D., Porporato M., Patetta A., Manino A. 2011. Toxicity of neonicotinoid insecticides to honey bees: laboratory tests. Bulletin of Insectology 64(1):107-113.

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Table 1. Analytical chemistry results.

			Concentration in ng/g				
Sample	Date				Thiametho		
ID	Collected	Sample type	Clothianidin	TZNG	xam	Other findings	
IL-004-	23 May					Imidacloprid <lod< td=""></lod<>	
01	2013	dead bees	7.9	2.9	<lod< td=""><td>Atrazine 11</td></lod<>	Atrazine 11	
IL-005-	23 May					Imidacloprid <lod< td=""></lod<>	
01	2013	live bees	<lod< td=""><td><lod< td=""><td><lod< td=""><td>Atrazine <lod< td=""></lod<></td></lod<></td></lod<></td></lod<>	<lod< td=""><td><lod< td=""><td>Atrazine <lod< td=""></lod<></td></lod<></td></lod<>	<lod< td=""><td>Atrazine <lod< td=""></lod<></td></lod<>	Atrazine <lod< td=""></lod<>	

Limit of detection = 0.5 ng/g for clothianidin, 0.4 ng/g for TZNG (a metabolite of clothianidin) and 0.6 ng/g for thiamethoxam, 1.3 ng/g for Atrazine, and 1.5 ng/g for Carbaryl.

Figure 1. Hives sampled for testing



Hive where sample was collected, prior to collection



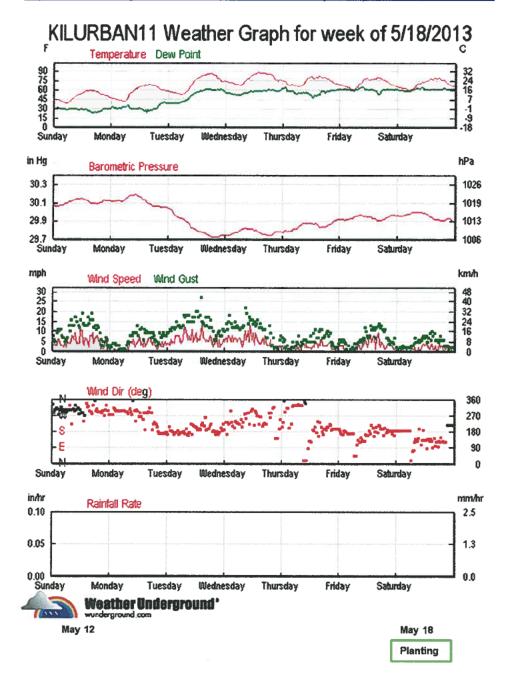
Sample of "odd behaving" bees clinging to grass in front of hive – bees collected for sample IL- 005-01

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Figure 2. Weather History for the three days before and after Sunday May 19

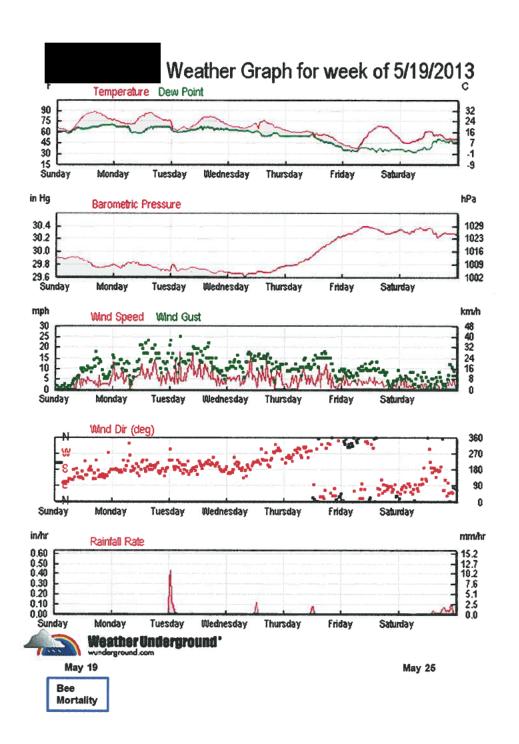
Data source:

http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=KILURBAN11



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Figure 3. apiary (top) and surrounding environment (bottom)



Apiary along North side of lot. Planted field is 40 feet behind Hives.



West hives in Apiary.

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Field view North of Apiary looking East



Field View Northwest of

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Figure 4. Apiary location and surrounding area. Imagery reported on Google Maps as from 2013 Champaign County GIS Consortium.



Final Report

Investigation of a May 8, 2013 Bee Kill Incident Purported to be Associated with Planting of Insecticidetreated Maize Seed near Maple Park, IL

Report Number

IL-

Guideline Requirements

None

Author

Jessica L. Walden-Gray

Completion Date

October 21, 2013

Submitter:

Bayer CropScience LP

2 T.W. Alexander Drive

Research Triangle Park, North Carolina 27709

1.0 Background

On May 29, 2013, Iain Kelly of Bayer CropScience (BCS) was contacted by Jerry Hayes of Monsar	to
regarding a report of large numbers of dead bees at hives located in an apple orchard at	
in Maple Park, IL. Dr. Kelly contacted on May 29 and arranged for a BCS	
representative to visit the site. is the beekeeper who manages the 52 hives that were affected	cted
on May 8 while pollinating 20-25 acres of apples at during corn planting in nearby field	ds. On
May 8, it was reportedly observed that as an adjacent field was being planted to corn, a large cloud	of
dust was blowing from the field into the orchard. Bee mortality reportedly occurred at the hives in th	е
orchard within 24 hours after this event. a Northern Illinois bee inspector, visited the	e site
and took photos, and collected samples of dead bees. estimated 33% total bee loss are	ıd
anticipated a full recovery. Mark O'Rourke of BCS visited the site on June 14 but did not obtain same	ples
for analysis.	

2.0 Investigative Actions

2.1 Field Methods

Mark O'Rourke visited the site on June 14 but did not collect samples since he was aware that sample had previously been collected by and sent to for residue analysis via the Illinois State

Department of Agriculture. Mr. O'Rourke contacted again on June 25 to determine whether the affected hives had recovered.

Pertinent daily weather data (temperature, humidity, rainfall, daily wind conditions, etc.) recorded at a weather station 9.2 miles NW of the bee yard were obtained from wunderground.com.

3.0 Results

3.1 Weather Conditions Prior to and During the Incident

Records from a nearby weather station in Sycamore, IL May 8 show that winds were blowing from the south in the morning and the southwest in the afternoon. Wind speeds were 0-3 mph with gusts up to 8 mph in the early afternoon. Temperature ranged from a low of 51°F to a high of 81°F. Daily humidity ranged from a low of 23% to a high of 81. In the three days before and after May 8, 0.76 inches of rain fell on May 5, no rain fell on May 8, and 0.18 inches fell on May 9.

The orchard and apiary would have been downwind from planting in that adjacent fields visible from satellite images (Figure 2).

3.2 Observations of Bee Mortality and Behavioral Impairment

Dead bees were reportedly observed at all 52 hives in the apiary by assessed that the hives experienced a 33% total bee loss.

3.3 Pesticide Residue Analysis Results

No results have been made available to Bayer CropScience

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3.4 General Health Status of the Hives Involved

anticipated full recovery of colonies without any hive die-off. reported that the hives had completely recovered as of June 25.

4.0 Discussion

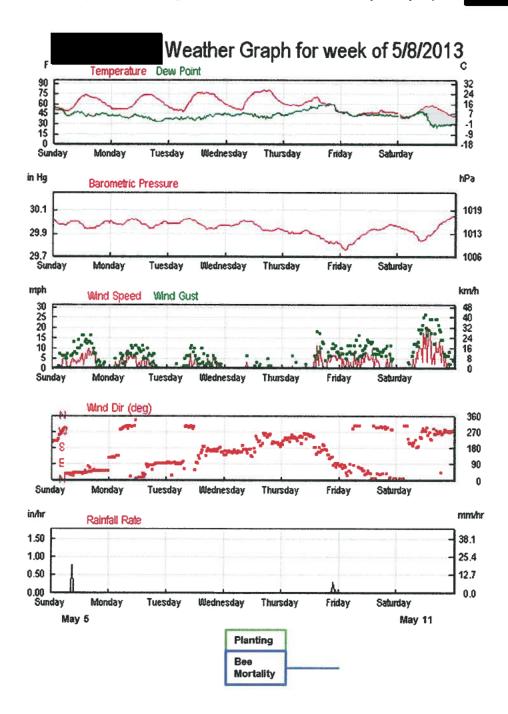
The circumstances reported suggest that bees in the orchard may have been exposed to toxic levels of a pesticide seed treatment that was present in abraded corn seed dust released during planting. Without further information including residue analysis, and details of pesticide applications to the apple orchards, it is not possible to determine the cause of the observed mortality.

The observed level of bee mortality, while clearly undesirable, did not appear to pose a serious risk of colony loss for the affected colonies. Colonies were strong enough to recover to normal strength within a few weeks.

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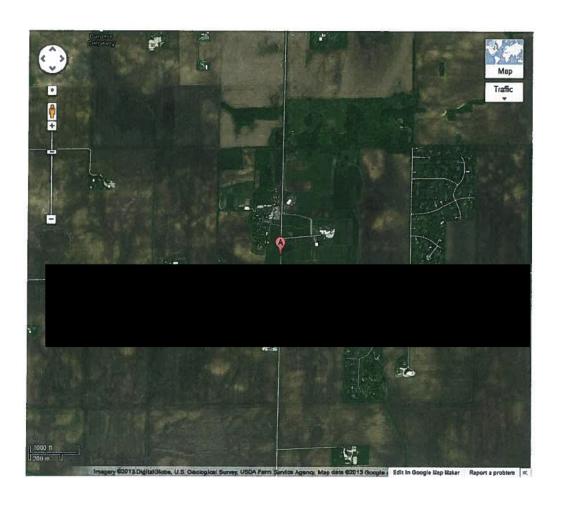
Figure 1. Weather History for the week of Wednesday May 8

Source: http://www.wunderground.com/weatherstation/WXDailyHistory.asp?ID=



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Figure 2. and surrounding area.



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